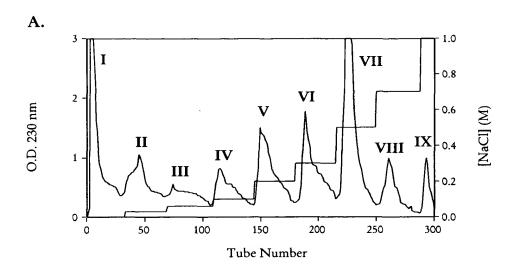


Fig. 1.0



B.

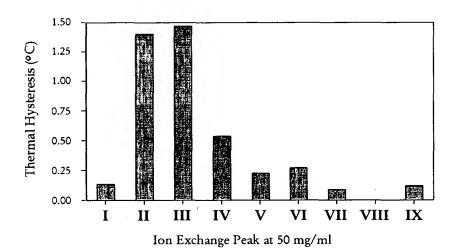


Fig. 1.1

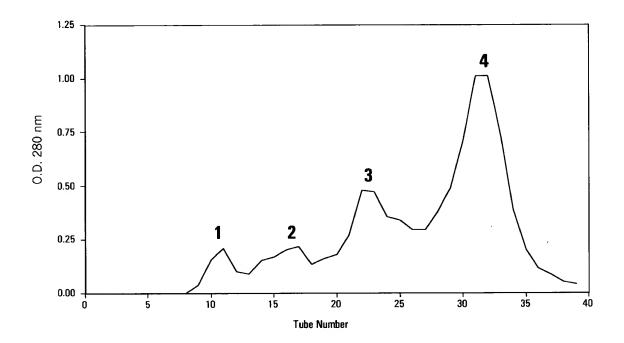
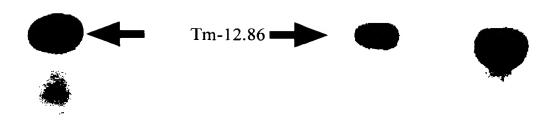
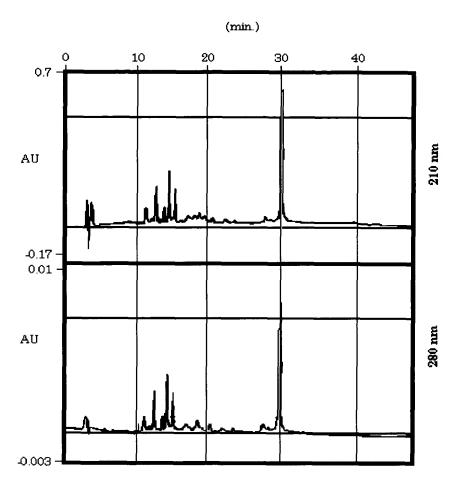


Fig. 1.2



12.5 25

Fig. 1.3 Fig. 1.4



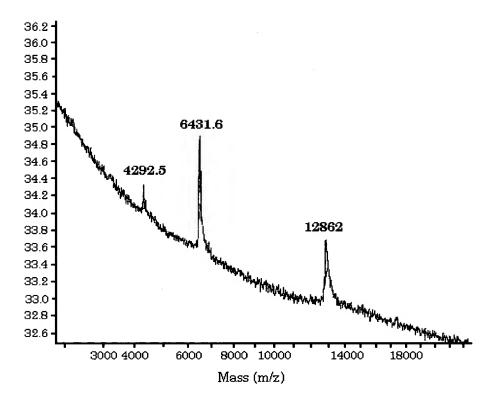
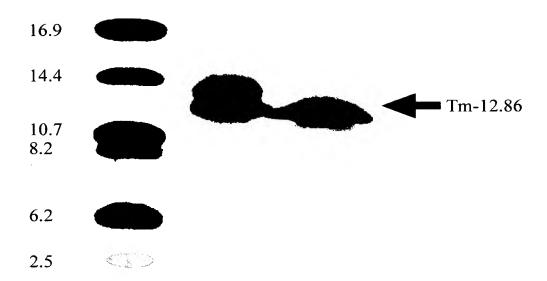


Fig. 1.6



w w/o

Fig. 1.7

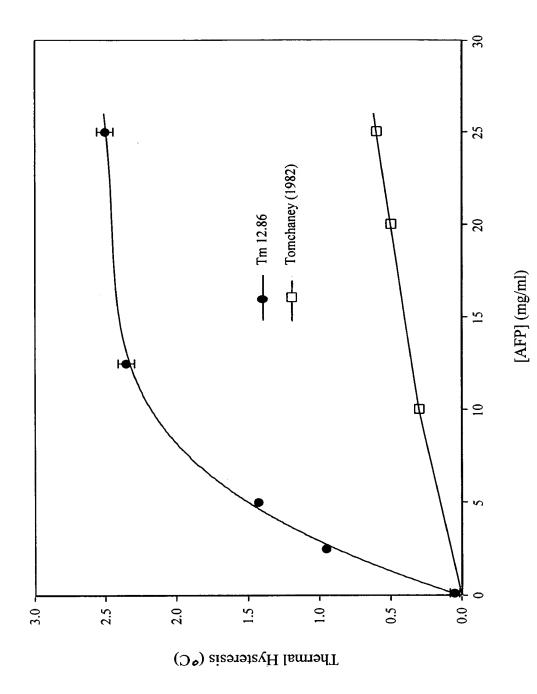


Fig. 1.9

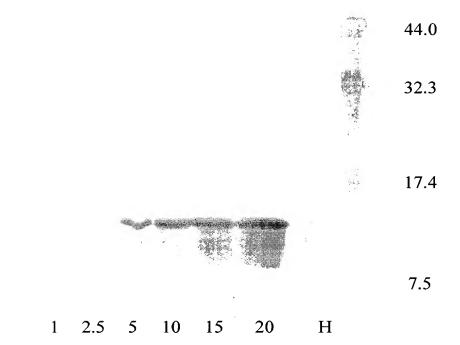


Fig. 1.10

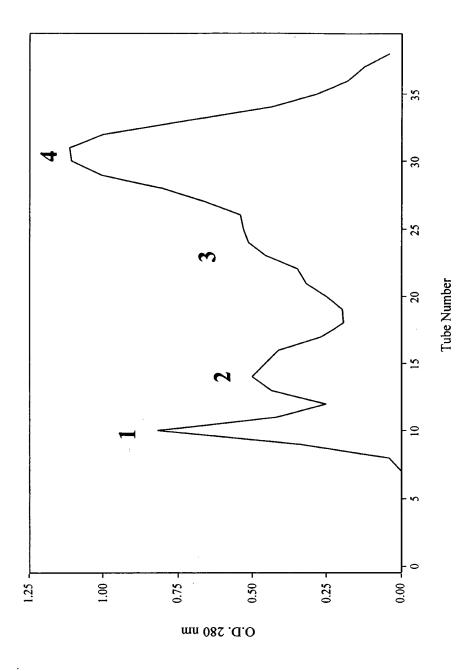


Fig. 1.11

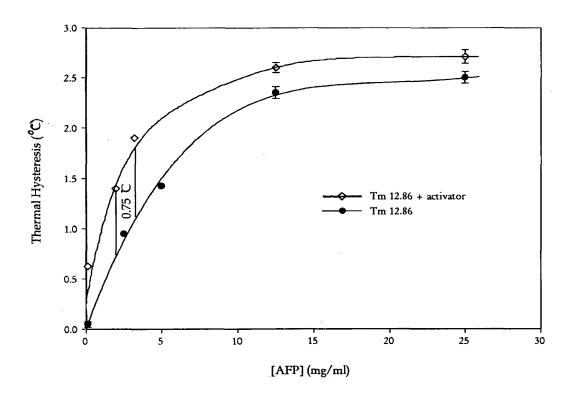


Fig. 1.12

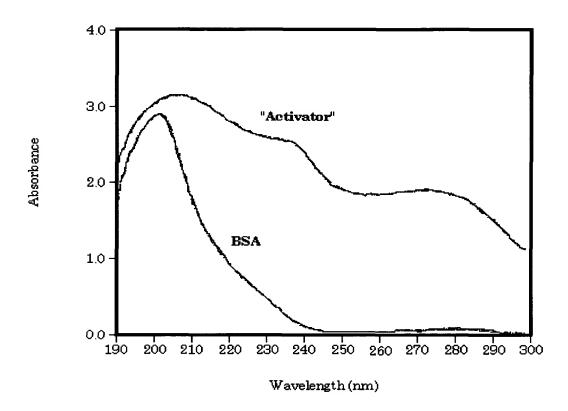


Fig. 1.13

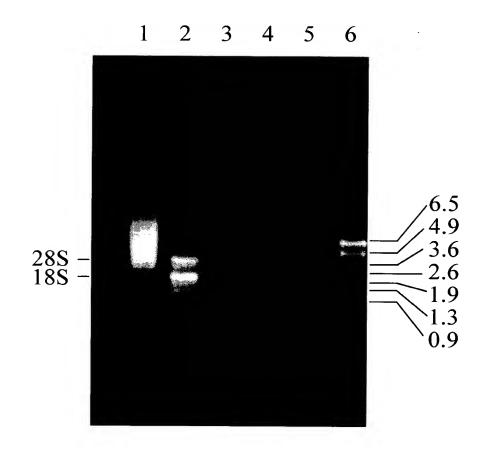


Fig. 2.0

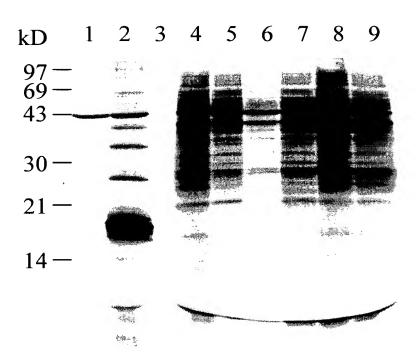


Fig. 2.1

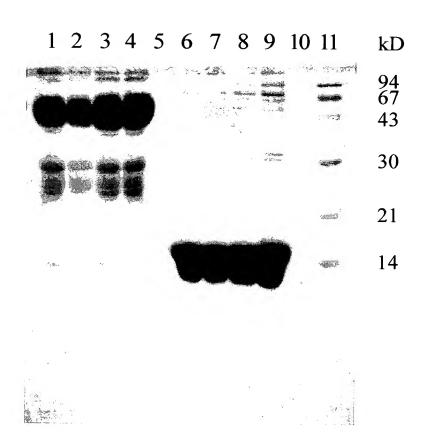


Fig. 2.2

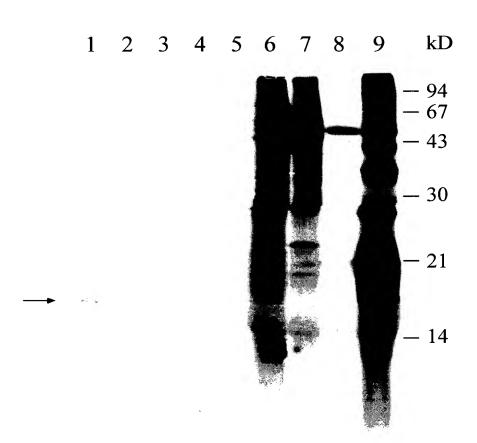
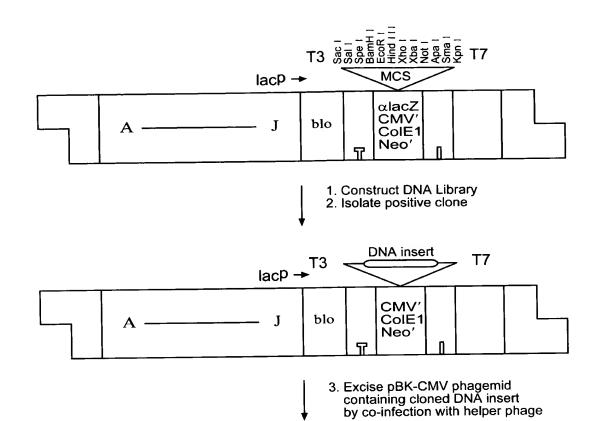
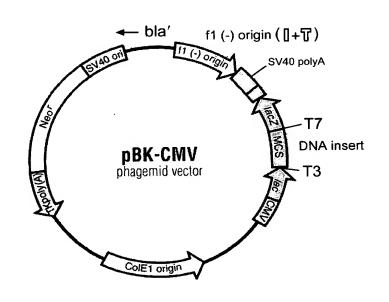
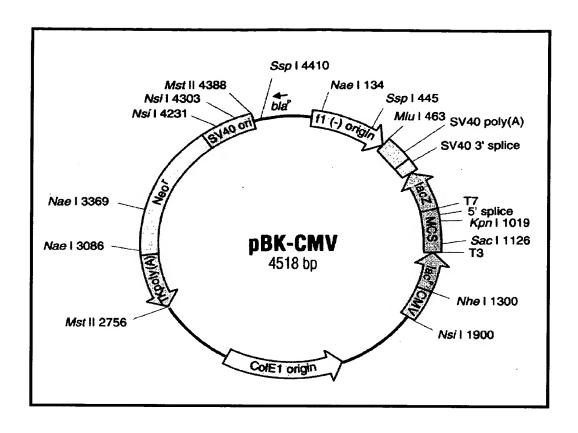


Fig. 2.3







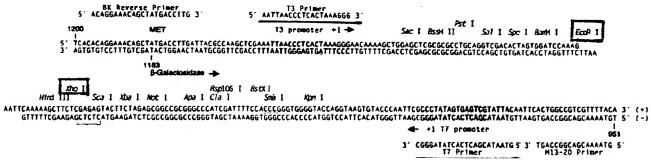


Fig 2.4b

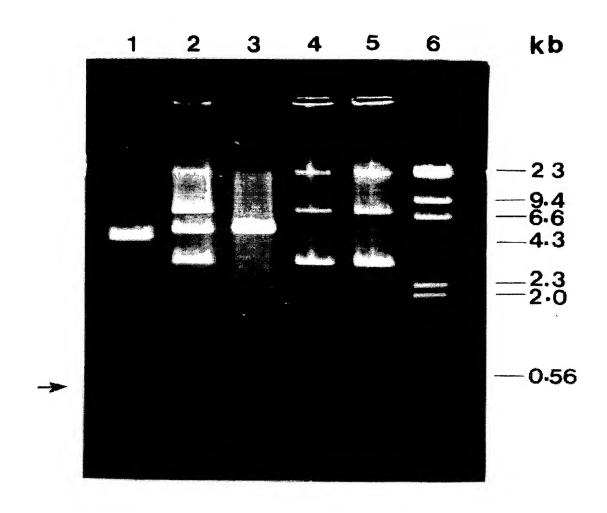


Fig. 2.5

DNA sequence of Tm 13.17 cDNA clone

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H R I I AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAAGTTGCTCTGTTGTCTAATCT M K L L C C L I S 61 CCCTCATTCTGTTGGTCACAGTTCAGGCCCTGACCGAGGCACAAATTGAGAAACCTGAACA L I L L V T V Q A L T E A Q I E K L N K 121 AGATCAGCAAAAAATGCAAAATGAAAGTGGAGTGCGCAAGAGATCATAACCAAAGCTC I S K K C Q N E S G V S Q E I I T K A R 181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA N G D W E D D P K L K R Q V F C V A R N 241 ACGCCGGTCTGGCCACGGAATCGGGAGAGAGTGTGTGAGGGGAGAAGGTGA A G L A T E S G E V V V D V L R E K V R 301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA K V T D N D E E T E K I I N K C A V K R 361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATGTGTCATGAAAAACCAAGCCAA D T V E E T V F N T F K C V M K N K P K 421 AGTTCTCACCAGTTGATTGAACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC F S P V D * 481 ATATAAAAAATAAAAGTGTTTCTGATGTAAAAAAAAAAA		. –			C																
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241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTCGACGTGTTGAGGGAGAAGGTGA A G L A T E S G E V V V D V L R E K V R 301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA K V T D N D E E T E K I I N K C A V K R 361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATGTGTCATGAAAAACAAGCCAA D T V E E T V F N T F K C V M K N K P K 421 AGTTCTCACCAGTTGATTGAACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC F S P V D * 481 ATATAAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAA	101	-																			
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h o I 481 ATATAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAA		F	S	P	V	D	*													*	
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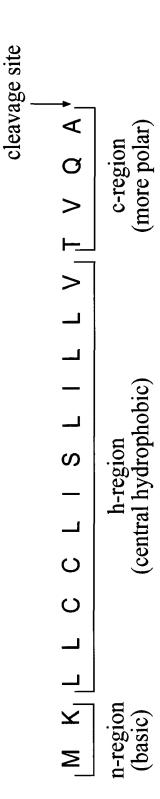


Fig. 2.66

A. Mature Tm 13.17 amino acid r sidure

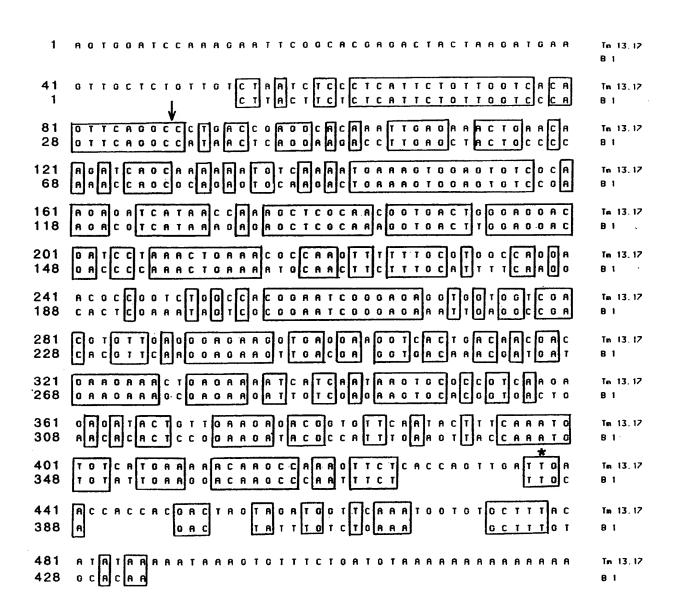
- 1 LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR
- 51 NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
- 101 TFKCVMKNKP KFSPVD

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala B = Asx C = Cys D = Asp E = Glu F = Phe G = Gly H = His I = Ile K = Lys L = Leu M = Met N = Asn P = Pro Q = Gln R = Arg S = Ser T = Thr V = Val W = Trp Y = Tyr	6 0 4 8 13 4 0 6 16 5 1 8 3 4 6 5 8 14 1	5.172 0.000 3.448 6.897 11.207 3.448 3.448 0.000 5.172 13.793 4.310 0.862 6.897 2.586 3.448 5.172 4.310 6.897 12.069 0.862 0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.



Tm 13.17	3	EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA 52	
AFP-3	1	ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA 50	
Tm 13.17	53	GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF 10:	2
AFP-3	51	GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF 10	0
Tm 13.17	103	KCVMKNKP 110	
AFP-3	101	KCVHDNRS 108	

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

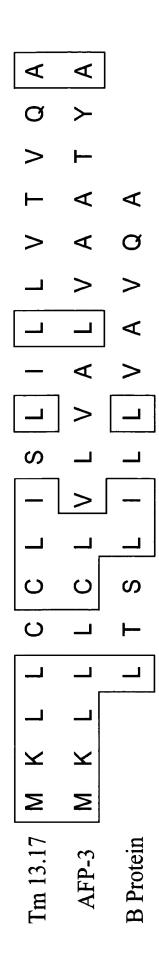
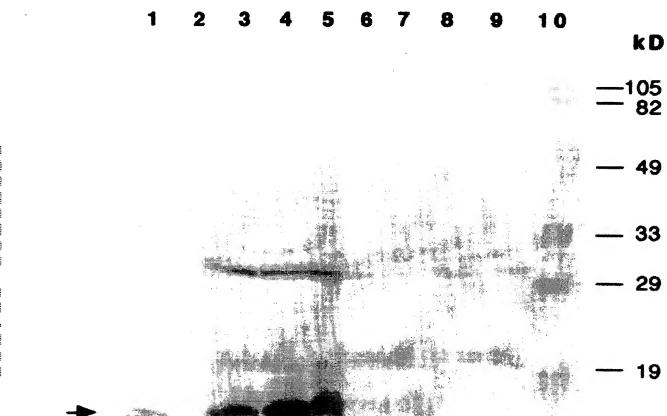
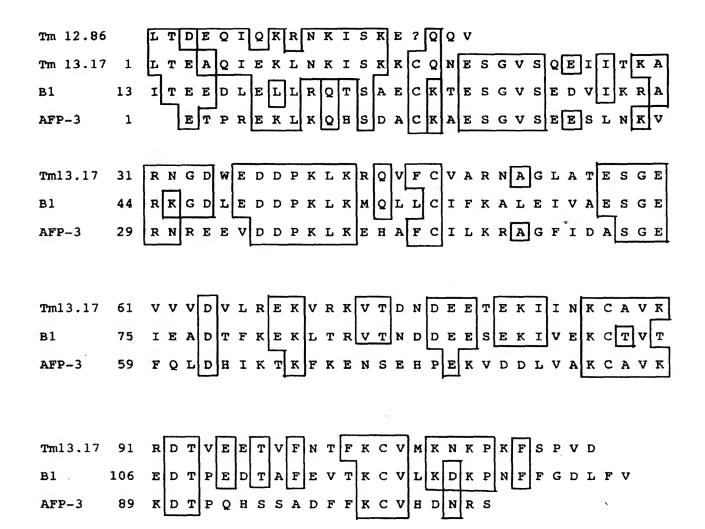


Fig. 2.9

Tm 13.17 NH2-L T E A Q I E K L N K I S K K C Q N E Tm 12.86 NH2-L T D E Q I Q K R N K I S K E ? Q Q V

Fig 2.10





1	GG	i C	Α	С	G	A	G	С	A	A	A	A	A M	T	G	A K	Α.	A	C L	T 	c	F C.	T :	C.	T L	T (G	T (3	r 0	T =	т	A	i C	G	F	T	С	G A	C		G C	; c
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26	GT V	G	G A	C		A T	С		G E			G A			G G			G D	A		A (C) 	A A	A 7		3 7 /	ΓG	à (G	G V			C			A K		A (СС	
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poly (A) tail

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poly (A) tail

Start

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T A A A G G T A C T A T C G T T A T G A A A A A

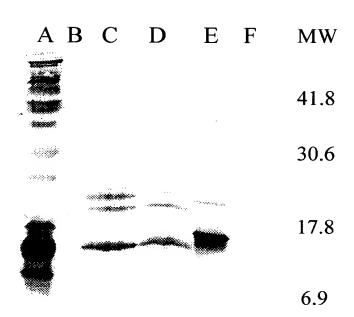
Predicted Amino Acid

Composition of 2-2 and 2-3

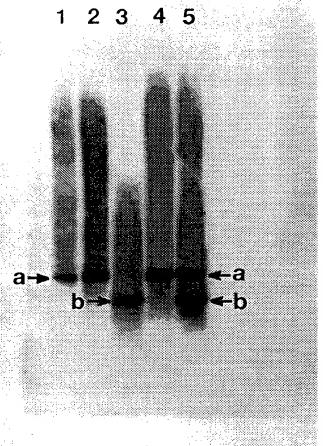
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Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Whole I fotell Composit	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lle	6	5.29	5.22
. K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	. 2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
∨ Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00







577 bp 483 bp

Fig. 4.0

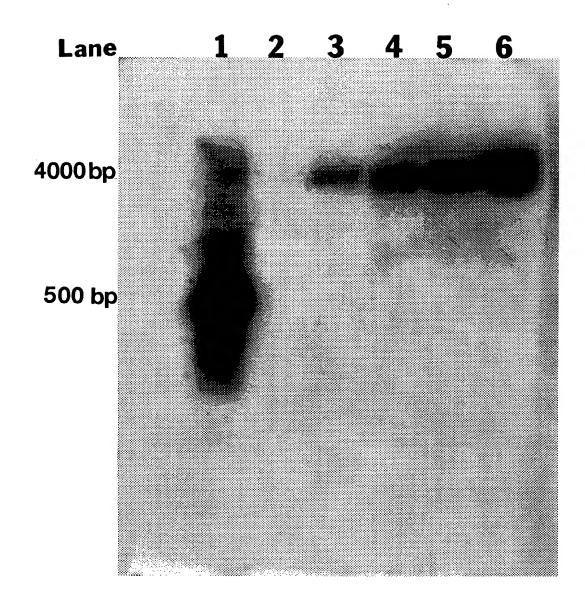
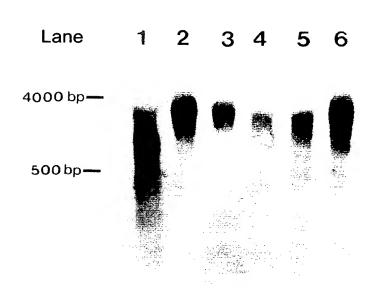
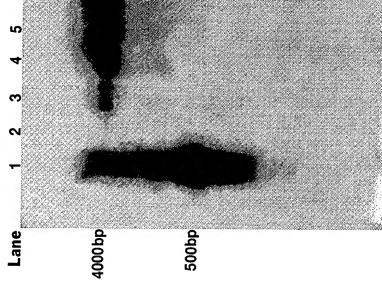
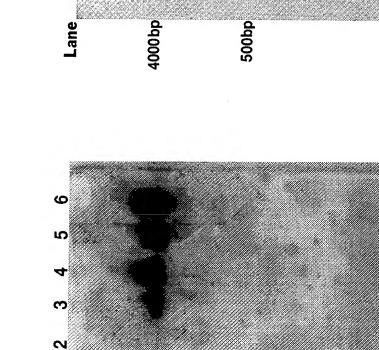


Fig. 4.1







4000b

Lane

Lane 1 2 3 4 5 6



Lane 1 2 3 4 5

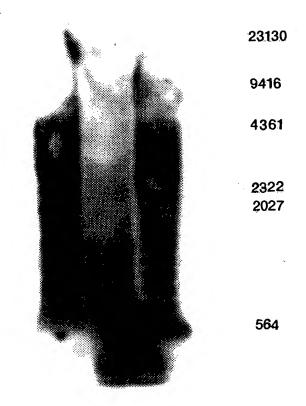


Fig. 4.5.

Tm 13.17 cDNA

7	AGTG	<i>ር</i> አጥ	CCB	BAG	ል አጥ	ም ፖር	GCA	CGA	GAC	TAC	TAA	GĀT	GAA	GTT	GCT	CTG	TTG	тст	AAT	СT
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121	AGAT	CAG	CAA	AAA	ATG	I.CV	AAA	TGA												
	I	S	K	K	C	Q	N	E	S	G	V	S	Q	E	I	I	T	K	A	R
181	GCAA	CGG	TGA	CTG	GGA	GGA	CGA	TCC	TAA	ACT	'GAA	ACG	CCA	AGI	TTI	'TTG	CGT	GGC	CAG	GA
	N	G	D	W	E	D	D	P	K	L	K	R	Q	V	F	C	V	A	R	N
		_																		
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241		_				E	S	G	F	v	v	v	ם	v	T.	R	E	K	v	R
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			e Pri																	`
361	GAGA	TAC	TGT	rTGA	AGF	GAK	GGI	GTI	CAA	TAC	TTT	CAA	ATC	TG1	CAT	'GAA	AAA	CAA	IGCC	'AA
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421							34347	, CA3.	-C-21		~ _ Z & X	<i>-</i>								
	73	_		**		-														

- polyadenylation signal poly (A) tail (26) 537 AGAGTATTCTAGAGCGGCCGGGGCCCATCGTTTTCCACCC

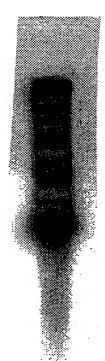
Forward Primer

2-2	LTDEQIQKRNKI SKECQQVS <mark>GVSQE</mark> TI DKVRTGVLV
Tm 13.17	LTEAQIEKLNKI SKKCQNESGVSQEII TKARNGDWE
B2	LTEEDLQLLRQTSAECKTESGASEAVI KKARKGDLE
AFP-3	ETPREKLKQHSDACKAES <mark>GVSEE</mark> SLNKVRNREEV
2-2	DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKH
Tm 13.17	DDPKLKRQVFCVARNAGLATESGEVVVDVLREKVRK
B2	DDPKLKMQLLC! FKALE! VAESGE! EADTFKEKLTR
AFP-3	DDPKLKEHAFC! LKRAGF! DASGEFQLDH! KTKFKE
	Reverse Primer
2-2	VAS DEEVDK! VQKCVVKKATPEET AYDTFKC! YDS
Tm 13.17	VTDNDEETEK! I NKCAVKRDTVEET VFNTFKCVMKN
B2	VTNDDEESEK! VEKCTVTEDTPEDT AFEVTKCVLKD
AFP-3	NSEHPEKVDDL VAKCAVKKDTPOHS SADFFKCVHDN
2-2	KPDFSPID
Tm 13.17	KPKFSPVD
B2	KPNFFGDLFV
AFP-3	BS

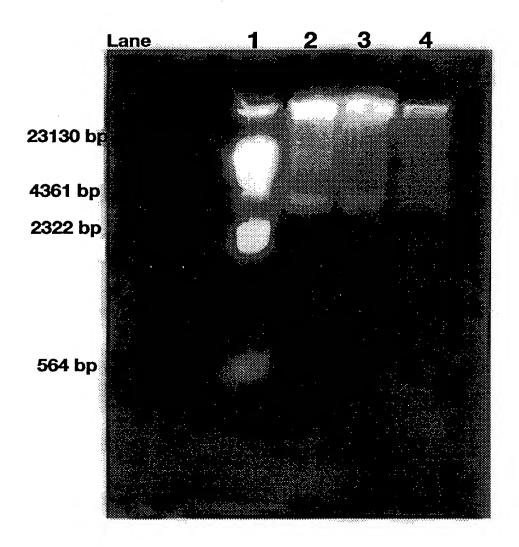
Fig. 4.6b

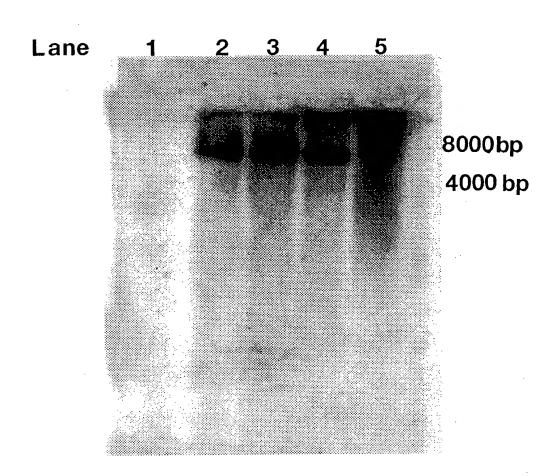
	percer	nt % c	omposit	ion	
Primer	A	С	G	T	MeltingTemperature(OC)
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

Fig. 4.6c



3600 bp





GGCACGAGCAAAAAACTCCTCTTGTGCTTTGCTTTCGCCGCC M K L L C F A F A A ATCGTCATCGGAGCTCAGGGCTCTCACCGACGAACAGATACAGAAA 47 AGGAACAAGATCAGCAAAGAATGCCAGCAGTGTCCGGAGTGTCCRNKISKECQQVSGVS 92 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT Q E T I D K V R T G V L V D D 137 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAACTGGAPKMKKHVLCFSKKTG 182 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCCVA TEAGDTNVEVLKA 226 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT Q K C V V K K A T P E E T A Y GACACCTTCAAGGTTATTTACGACAGTAAACCTGATTTCTCCCTD T F K V I Y D S K P D F S P 361 ATT GATT AATT GTTTTT GACT GAATTTT GAC AATAAA GGT polyadenylation signal 451 ACTATCGTTATGTAAAAAAAAAAAAAAAAAA

poly (A) tail

Predicted Amino Acid

Composition of 3-4

Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole Protein Co.	mposition Analysis
-------------------	--------------------

Whole Protein Compositi	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
FPhe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lle	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
QGIn	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
∨ ∨al	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 4.10b

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poly (A) tail

Fig. 4.11a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	` 9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I lle	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
TThr	9	7.07	7.83
∨ ∨al	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

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poly (A) tail

Predicted Amino Acid

Composition of 7-5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I lle	6	5.29	5.22
KLys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
TThr	9	7.08	7.83
∨ Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
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Fig. 4.14

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	(kDa)	(#)								aloondom fii) in charge
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2-2	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0		0	0	2.43	18.0 4.75		7.08	32.23
2-3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0		0	0	2.43	18.0 4.75		7.08	32.23
3-4	12.84	115	2.41	3.02	3.44	5.29	9.11	1.02	4.41	28.04	1.78	3.32	2.54	3.32 2.54 2.14	0	0	0	2.43	18.0	4.75	7.09	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	3.31 2.54 2.13	0	0	0	3.64	6.91	4.74	7.07	32.38
7-5	12.84 115 3.21	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	1.78 3.32 2.54 2.14 0	0	0	0	2.43 18.0 4.75	0.8	4.75	7.08	32.23



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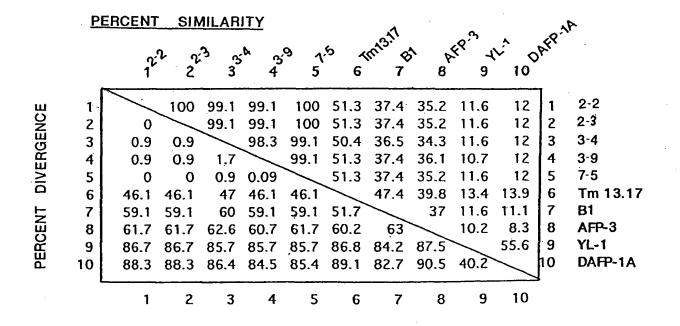
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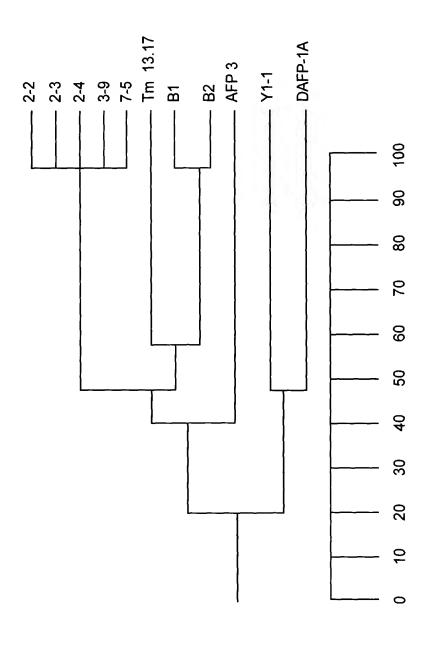


NUCLEOTIDE SEQUENCES

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Ц	6	36.9	36.4	37.2	36.4	37.1		57.2	37.4	23.3	21	6	Tm 13.17
F	7	39.9	41.2	41.7	40.7	41.2	30.2		39.3	21.9	22.1	7	B1.
PERCENT	8	41.8	42.4	43.1	42.3	42.3	45.3	49.4		21.8	23.1	8	AFP-3
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8	10	51.4	62.7	46.4	46.5	46.5	60	58.1	61.8	35.5		10	DAFP-1A
		1	2	3	4	5	6	7	8	9	10		

AMINO ACID SEQUENCES





% Nucleic Acid Identity

Fig. 4.20

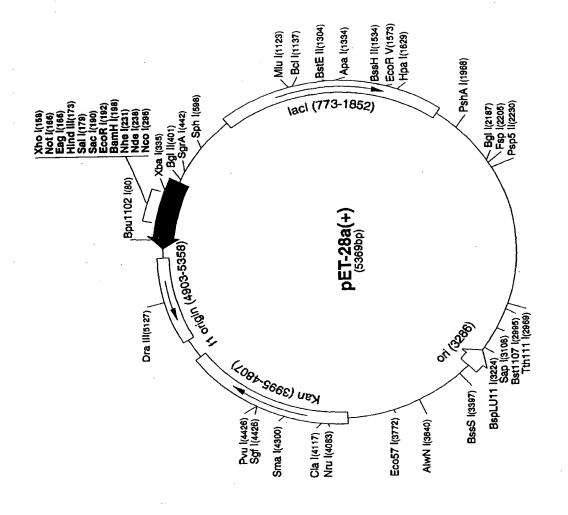
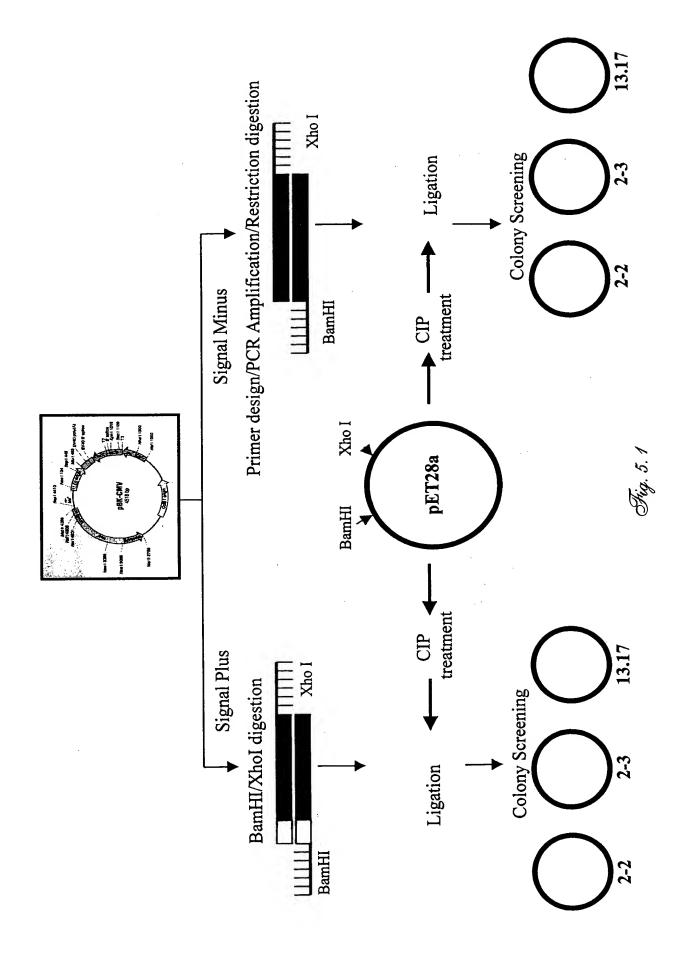
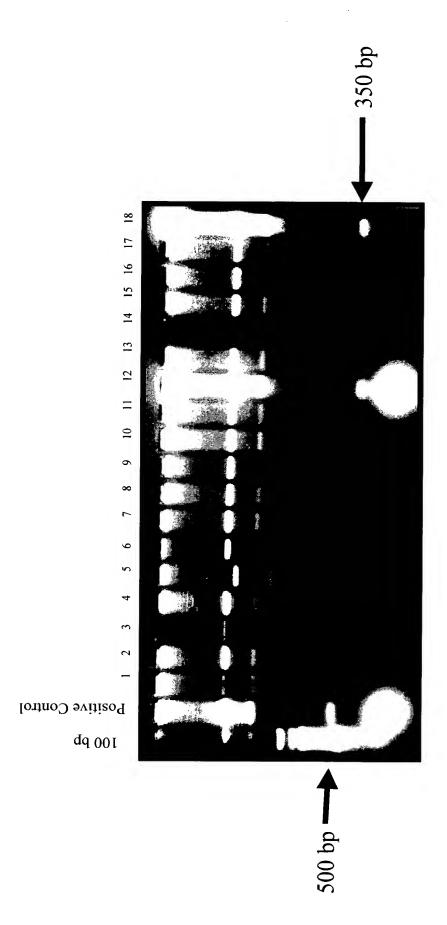
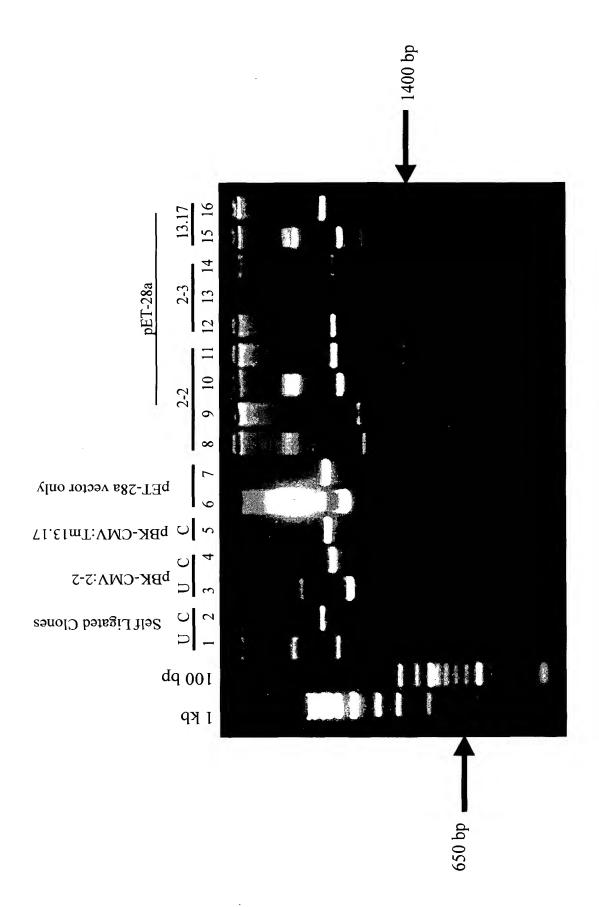


Fig. 5.0





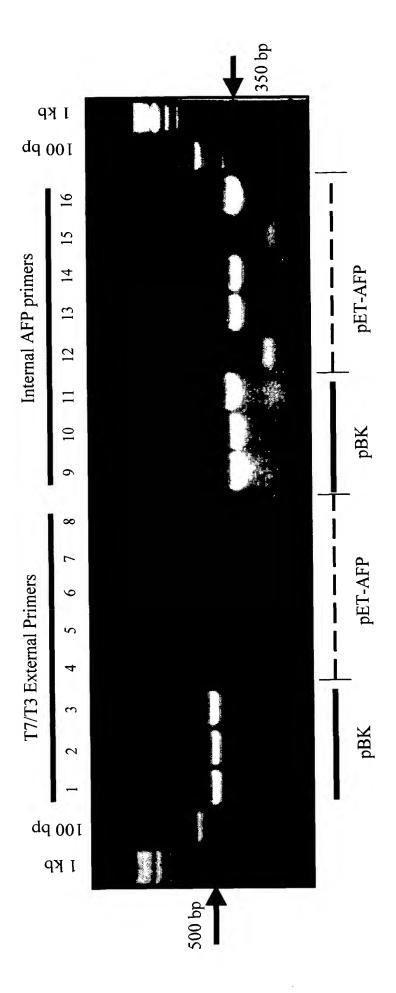
Hig. 5.2



GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC

M K L L C F A F A A ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA AGGAACAAGATCAGCAAAGAATGCCAGCAGTGTCCGGAGTGTCCRNKISKECQQVSGVS 92 CAAGA GACGAT CGACAA GT C CGCACAGGT GT CTT GGT CGAT GAT Q E T I D K V R T G V L V D D 137 CCCAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAACTGGA P K M K K H V L C F S K K T G GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCCVA TEA G D T N V E V L K A 226 AAGCTGAAGCATGTGGCCAGCGACGAAGATCGTGKLKHVASDEEVDKIV CAGAAGTGCGTGGTCAAGAAGGCCACAGAGGAAACGGCTTAT Q K C V V K K A T P E E T A Y GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCTD T F K C I Y D S K P D F S P 361 ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT 406 polyadenylation signal ACTATOGTTATGTAAAAAAAAAAAAAAAAA 451

poly (A) tail



Hig. 5.4

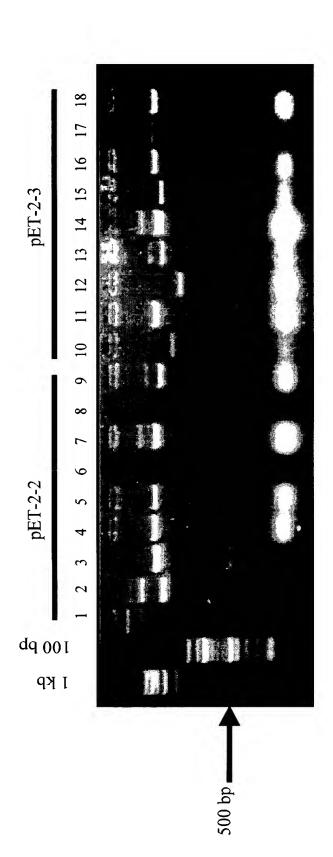


Fig. 5.5

pET-28a Recombinant Histidine cleaved AFP

His-tagged Clone 2.2 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -40 -35	141
AFP Start GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG G1y G1n G1n Met G1y Arg G1y Ser G1u Phe Ala Arg Ala Lys Met -30 -25 -20	Codon 186
AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -10 -5	231
N-terminal of mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser 1 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75 80 85	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
Stop Codon ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTAATA TCGTTATGTA AAAAAAAAAA	645
AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT	681

His	-tag	ged (clone	e 2.2	2 wit	thou	t sig	gna 1	sequ	uenc	е				
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						GGC Gly									141
						GGA Gly		CTC	ACC	GAC		CAG	ATA	CAG	186
						AAA Lys									231
						AAA Lys									276
						CAC His									321
						GGA Gly									366
						GCC Ala									411
						AAG Lys									456
						ATT Ile									501
	ATT Ile		TAA	COC CTCC		ACC A	ACCA(CCAC(CA CO	CACTO	GAGA1	ſ			543

His-tagged clone 2.3 with signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -40 -35	141
AFP Sta GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys <u>Met</u> -30 -25 -20	rt Codon 186
AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -10 -5	231
N-terminal of Mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser 1 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75 80 85	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TIGTTITGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTACTA TCGTTATGAA AAAAAAAAAA	645
AAAAAAACTC GAGGACCACC ACCACCACCA CTGAGAT	682

His.	-tago	ged (Clone	2.3	3 wit	chout	t sig	gna I	sequ	uence	5				
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								TGC Cys							231
								CGC Arg							276
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								ACC Thr							366
								GAC Asp							411
								GCC Ala							456
								GAC Asp							501
	ATT Ile		TAA	COC		ACC A	ACCAC	CCACC	CA CO	CACTO	GAGA⊺	Γ			543

His-tagged Tm 13.17 with signal sequence	
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -65 -60 -55	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -50 -45 -40	141
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile -35 -30 -25	186
AFP Start Codon CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser -20 -15 -10	231
N-terminal of mature AFP CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT Leu Ile Leu Leu Val Thr Val Gln Ala -5 N-terminal of mature AFP CTC ATT CTG TTG GTC ACA GTT CAG GCC TTG ACC GAG GCA CAA ATT Leu Ile Leu Leu Val Thr Val Gln Ala 5	276
GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly 10 15 20	321
GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu 25 30 35	366
GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC ASp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn 40 45 50	411
GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu 55 60 65	456
AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu 70 75 80	501
AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu 85 90 95	546
ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys 100 105 110	595
Stop Codon TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG Phe Ser Pro Val Asp * 115	643
Polyadenylation signal Poly-A tail TGTGCTTTAC ATATAAA <u>AAT AAA</u> GTGTTTC TGATGTAAAA AAAAAAAAAA	693
AAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT	743
TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT	777

His-tagged Tm 13.17 without signal sequence								
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG 50								
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -30 -25	96							
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -20 -15 -10	141							
N-terminal of mature AFP GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys -5 1 5	186							
CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser 10 15 20	231							
CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp 25 30 35	276							
CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly 40 45 50	321							
CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu 55 60 65	366							
AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile 70 75 80	411							
ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val 85 90 95	456							
TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser 100 105 110	501							
Stop Codon CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT Pro Val Asp * 115	543							

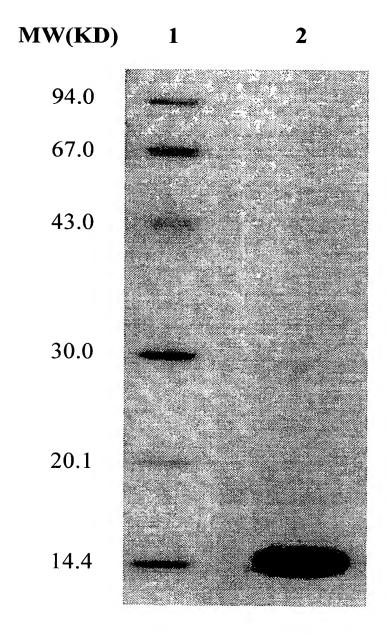


Fig. 6.0

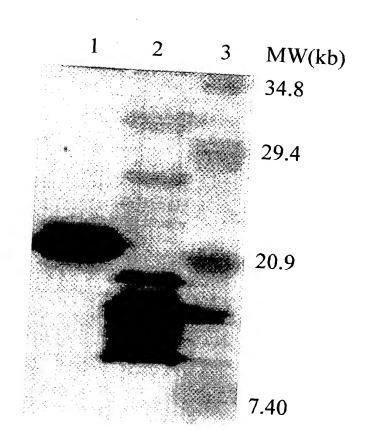


Fig. 6.1

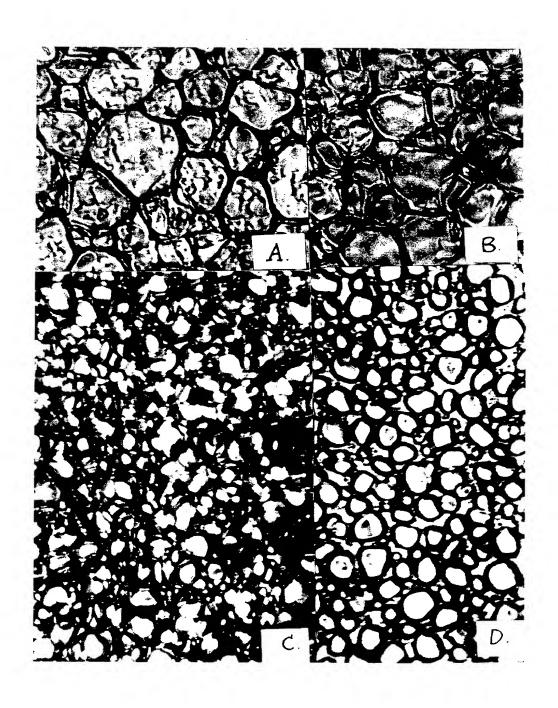
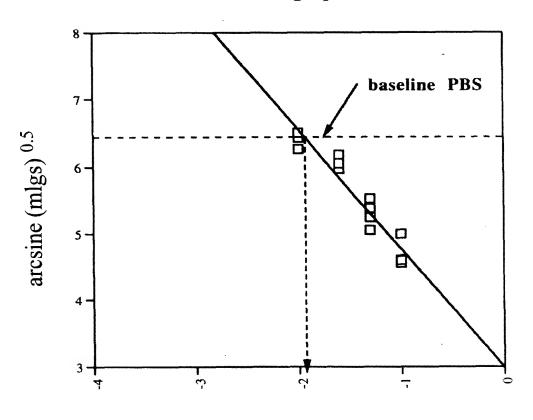


Fig. 6.2

Tm 13.17 S-graph data

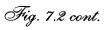


log dilution

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
Α	Alanine	Ala	aliphatic	mod. hydrophobic	low
В	Asp or Asn	Asx			
С	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
E F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
н	Histidine	His	basic, imidazole	highly hydrophilic	high
1	Isoleucine	lle	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
0	, ,				
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gln	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
Ü					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X	, ,				
Υ	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			
		ACD	Any Acidic		
		ALP	Any Aliphatic		
		ALH	Any Aliphatic Hydroxyl		
		ARO	Any Aromatic		
		BAS	Any Basic		
		HY-	Hydrophobic		
		HY+	Hydrophilic		

Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of Tm12.84	Tm13.17	Concensus with Tm 13.17	B1	Concensus with B1	AFP-3	Concensus with AFP-3
1 2	Å C	A C	Ĉ.	A C	A C	A C	A G·	A		A N	C A	N
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5	G	A G	Ĝ	G	G	G	T	N		N	T	N
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12 13	A	• A	A	Α .	A	 A	Ā	•	Α?	•	Ā	A
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15 16	G A	G A	G A	G A	G A	G A	G A	G A	G?	G A	G A	G A
17 18	A A	A	A A	A A	A A	Â	A G	A R		A R	A G	A R
19 20	C T	C	C T	C T	Ç	C T	T T	Y T		Y T	C T	Y T
21	c	C C	C C	C	c	c	G	C/G C		c/G C	C	C/G
22 23 24	T C	Ť C	Ť C	Т	Ť	т	т	Т		T	Ť	C T
25	Т	T	т	C T	Ť	C T	C T	С Т		C T	C C	Ç
26 27	T G	T G	T G	T G	G G	T G	G T	N N		2	T C	N N
28 29	T G	T G	T G	Ť G	T G	T G	Ť G	Ť G		T G	T G	Ť G
30 31	C T	C T	C T	C	C T	C T	C	Y	С	Y	T C	Y
32 33	T T	T T	Ť	T T	Ţ	T T	T A	T T/A	Ť T	T T/A	T T	T T/A
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39 40	C G	C G	C G	C G	C G	C G	C	G/C C	C C	G/C Y	C G	e/c Y
41 42	c	C C	C C	c c	c	c c	C	Y C	T C	Y C	Ţ	Y
43 44	G C	G C	G C	G C	G	G C	A T	R Y	A T	R Y	G C	R Y
45 46	C A	C A	C A	C A	C A	Č A	Ť C	Ý	T C	Ý	C T	Ý N
47 48	Ť C	Ť C	Ť C	Ť	Ť	T	T	T	Т	Ť	T	T
49	Ġ	G	G	G	G	e C	G T	C/G N	G T	C/G N	G G	C/G N
50 51	T C	Č.	Č	T C	C	r C	T G	T C/G	T G	T C/G	T C	T C/G
52 53	A T	A T	A T	A T	Ť	A T	G T	R T	G T	R T	G C	R Y
54 55	c G	c G	C G	C G	C G	C G	C A	C R	C	C N	C G	0
56 57	G A	G A	G A	G A	G A	Ğ A	C A	G/C A	Č A	G/C A	Ċ G	G/C R
58 59	G	Ğ	G	G	G	Ğ	G T	G Y	Ğ	Ĝ Y	A C	R Y
60 61	7	T	T	T	T	T	T	T	Ť	T	С	Y
62	C A	C A	C A	C A	C A	C A	C A	C A	C A	C A	T A	¥ A
63 64	G G	G G	G G	G G	G G	G G	G G	G G	G G	G G	C G	N G
65 66	C T	C T	C T	C T	C T	C T	C	C Y	C	C Y	C	C Y
67 68	C T	C T	C T	C T	C T	C T	C T	C T	A T	N T		N T
69 70	C A	C A	C A	C A	C A	C A	G A	C/G A	A	N A		N A
71 72	c	c	C	C	C	C	C	C C	C	C Y		Č Y
73 74	G A	G	G	Ğ A	Ğ	G	G	Ğ A	c	G/C	G	G/C
75 76	C	A C	A C	T	Ĉ	A Y	A G	N	A G	A N	A	Ą
77	G A	G A	G A	G A	Ā	G A	G C	G N	G A	G N	A C	R N
78 79	A C	A C	A C	Å C	A C	Ĉ	A C	Č	Ğ	c/G	A C	A C/G
80 81	A G	A G	A G	Å G	A G	A G	A	A R	A C	A N	C T	N N
82 83	A T	A T	A T	A T	A T	A T	A T	A T	C	N T	G	N N
84 85	A C	A C	ê C	A C	A C	A C	T G	A/T C/G	T G	A/T C/G	T G	A/T C/G
86 87	A G	Å G	Å G	A G	A G	A G	Ā G	A G	A G	A G	Ā	A G
88 89				Ä	A					N		Ň
90	Ä	Â	Ã	A	A	Â	Ã	A A N	Å	A	Ğ	R
91 92 93 94 95 96 97 98 99	Ĝ	Ĝ	Ĝ	A A G	A A G	Ğ	Ť	N	Ť	N N	T	N
93	A	A A	A	G A A C	G A	G A	A	G A	G Ç	G N	G A	G N
95 96	Ĉ	A C	A C	Â C	A C	A C	A C	A C	C	N C	A G	Ņ
97 98	A	A	A	A	A	A	A A	A A	C	N A	C A	N
99 100	G A	G A	G A	G	G A	G A	G A	G	A	R	G	R
101 102	Ť	Ť	Ť	A T	Ť	Î	Ī	Î	Ĉ	Ŷ	Ä	N N
103	Ä	Ā	Ā	C A G	Ä	Ä	Ä	Ä	Ā	A	A	A A
103 104 105	Ç	Ç	Ç	G C	G C	G C	G C	G C	G C	G C	G C	G C
106 107	A	A	A	C A A	A	A	A	G A A C A A G A T C A G C A A A R	C T A C T G C C C A A A C C A G C G C A G A G T	A/T A N N G N N C N A R A Y C A G C R N A R A R T	A A G C T G A A G C A G C A C G C C C T	R N
108 109	A G	A G	A G	A G	A G	A G	A	A R	A G	A R	C G	N R
110 111	A	A	A	A	A G	A R	A	A R T	Á	A R	ć	N N
112 113	T G	T G	Ţ	A T	Ť	T G	T G	T G	Ĩ	Ť	Ť	Ť
114	č	č	Ċ	č	č	000	ĭ	G Y C	C	G Y	G C	Y N.
116 117	A A A A G G A A C C A A G G A A T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A A T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A A T G C C A G	G C C A G	G A A C A A G A T C A G C A A A G A G T G C C A G	A A A A G G A A C A A G A T C A G C A A A G A R T G C C A G	A A C T G A A C A A G A T C A G C A A A A A T G T C A A	A R	G C A G	N A R	A A G	\$ R N N G N N Y N A R N N N O A GO C R N N R N N N T T T T T T T T T T T T T
117	G	G	u	G	G	G	*	к	G	ĸ	G	R

Position 118	Tm12.84-2.2 C	Tm12.84-2.3 C	Tm12.84-3.4 C	Tm12.84-3.9 C	Tm12.84-7.5 C	Concensus of C	Tm13.17 A	Concensus with N	B1 A	Concensus with N	AFP-3 G	Concensus with N
119 120	A G	A G	A G	A G	A G	Å G	A T	A N	C T	N N	C T	N
121 122	G T	G T	G T	G A	G T	G T/A	G A	G T/A	G A	G T/A	G A	G A/T
123 124	G T	G T	G T	G T	G T	G T	A	R T/A	A	R T/A	G T	R T/A
125 126	C C	C	C C	C	c C	c	G T	C/G Y	G T	C/G Y	C T	A CAG
127 128	G G	G G	G G	G G	G G	Ģ G	G G	G G	G G	G G	G G	G G
129 130	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G
131 132	T G	T G	T G	T G	T G	T G	T G	T G	T G	T G	T A	T R T
133 134	T C	T C	T C	T C	T C	T C	T C	T C	t C	T C	T C	r c
135 136	Ċ	C	c	C C	C C	c c	G C	c/G c	C G	C/G C/G	T G	N C/G
137 138	Ā	A A	A A	A	A	Â	A A	Â	À	A	A	A
139 140	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A
141 142	G A	G A	G A	G A	G A	G A	G A	G A	C G	G/C R	G T	G/C N
143 144	Ĉ	Ĉ G	Ĉ G	Ĉ G	Ĉ	Ĉ G	Ť	G/C Y	Ť C	G/C	Ċ	G/C Y
145 146	Ā	A T	Ā	Ä T	A T	Ä	Ā	Ā	Ā	A T	Č T	N T
147	Ċ	Ċ	Ċ	Ċ	c G	Ċ	À	N R	À	N R	Ċ A	N R
149	A	Α	A	Α.	A C	A C	ĉ	N C	Â	N C/G	â	N C/G
150 151	C A	C A	C A	C A	A	A	Ā	A	A G	A R	A	A R
152 153	A	A	A	A	A	A	A	A A	A	A	A G	R
154 155	G T	G T	G T	G T	G T	G T	G C	G Y	G C	G Y	G T	G Y Y
156 157	C C	c	c	c	c	c	C C	ç	Č	ç C	C C	C
158 159	G C	G C	G	G C	G C	G C	G C	G C	G C	G C	G C	G C
160 161	A C	A C	A C	A C	e C	A C	Â	ñ	Â	A N	A	Â
162 163	A G	A G	A G	A G	A G	A G	c G	N G	A G	N G	c c	G/C N
164 165	G T	G T	G T	G T	G T	G T	G T	G T	G T	G T	G T	G T
166 167	G T	G T	G T	G T	G T	G T	G A	G T/A	G A	G T/A	G A	G T/A
168 169	C T	C T	C T	C T	C T	C T	C T	C T	C T	C T	A G	N N
170 171	T G	T G	T G	T G	T G	T G	G G	N G	T G	N G	A	N R
172 173	G T	G T	G T	G T	G T	G T	G A	G T/A	G A	G T/A	G T	G T/A
174 175	C G	C G	C G	c G	C G	c G	G G	c <i>i</i> G	G	C/G G	G G	C/G G
176 177	A T	A C	A C	A C	A C	A Y	A C	Ŷ	A C	A	A C	Ą
178 179	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A
180 181	T C	T C	T C	T C	T C	r C	T C	T C	c c	Y C	c	Č
182 183	c	C	c c	c c	C	C	Ċ	Ċ	C	C Y	c	C Y
184 185	A	A A	Ä	A	A	A	A	A	A	A	A	A
186 187	A	A	A	A	A	A	A C	A	A C	A N	A C	A N
188 189	Ť G	T G	Ť G	T G	T G	T G	Ť G	T G	T G	Ť G	T G	Ť G
190 191	A A	Ä	Ā	Ä	Ā	Ā	Ā	Ā	Ā	Ā	Ā	A A
192 193	G A	G A	G A	G A	Ģ A	Ğ A	Å	R N	A	R N	A G	R N
194 195	A G	A G	A G	A G	Ā	A G	Ğ	R G/C	T G	N G/C	A G	N G/C
196 197	Č A	Č A	Č A	Č A	Č A	C A	C A	C A	C A	C A	C A	C A
198 199	C G	C G	C G	C	C G	C G	A G	N G	A C	N G/C	T G	N G/C
200 201	T C	T C	T C	T C	T C	T C	T T	T Y	T T	T Y	C T	Y
202 203	Ċ T	C T	Ċ T	Č T	C T	C T	T T	Y T	C T	Y T	T T	Y T
204 205	Ċ T	Ċ T	Ċ T		_	_	Ţ	Ý T	Ť	Y T	C T	Y T
206 207	G C T	G C T	G C T	C T G C	C T G C T T C	C T G C T	G C	G C	G C	G C	G C	G C
208 209	Ť	T T	Ť	T T	Ť	Ť	G T	N T	A T	N T	G C A T C	N T
210 211	Ċ	Ċ T	Ċ	Ċ	Ċ	T C T	G	C/G	Ť	N	C T	N N
212 213	T C T C G	Ċ G	T C T C G A A G	T C T C G	Ċ	Ċ	Ċ	N C G/C A R G	Ť C	N Y G/C	Ť	Y GIC
214 215	A A G	A A G	Ā	A A G	C G A A G	A A G	Ä	A	A A G	A	Ā	A
216 217	Ĝ	Ĝ	Ĝ	Ĝ	Ĝ	Ğ	Ğ	Ğ	Ğ	Ğ	Ğ	Ğ
218 219	A A A	A A	A A	A G A C T	A A A C T	A R A A C T G G A G T G	G C G T G G C C A G G A A C G C C G G T C T G G C C A C G G	A R N	G C A C T C G A A	A R G R N N	T G A A G A G C C G G A T T C G A C G	G C R T R R Y G A R G R R R R R Y Y G R T R T R R Y R R R G G
219 220 221	Ä	A A C	Â	Ä	Â	Ä	G	R	ç	Ņ	Ğ	Ņ
222	A C T	T	A C T	Ť	Ť	Ť	00	R C Y	ċ	Ý	Ö	Ý
222 223 224	G	G G	G	G	G	G	G	6	A	R	G	R
225 226 227	G G A G T	A G T	G G A G T	<u>A</u>	Ğ	Ğ	Ċ	G G A/T G/C T G	A A T	N YYGR ATN IR GY	Ţ	ñ
228	e L	G	G	G G	G G	ē	e I	Ġ	A .	R R	Ċ	N
229 230	G C	G C	G	G C	Ģ Ç	G C	G C	G C	A G T	G Y	A T	R Y
231 232 233 234 235	A C C G	A	0 C A A C C G	G G A G T G G C A A C C G	G G A G T G G C A A C C G	G C A A C C C G	C A	N	С	N R C C/G	C G	N R
233 234	C	A C C G	c	C C	c c	Ç	G G	A C C/G G	G G G	C C/G	ĉ	N C/G
235	G	G	G	G	G	G	G	G	G	G	G	G



Do at												
Position 236 237	Tm12.84-2.2 A A	Tm12.84-2.3 A A	Tm12,84-3,4 A A	Tm12.84-3.9 A A	Tm12,84-7.5 A A	Concensus of A A	Tm13 17 A A	Concensus with A A	B1 A A	Concensus with A A	AFP-3 C C	Concensus with N N
238 239	G C	G C	G C	A G C	G C	G C	T C	N C	Ť	Č K	Ã	N C/G
240 241	C G	C G	C G	C G	C G	C G	G G	C/G G	G G	C/G G	T G	N G
242 243	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G T	G A/T
244 245	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A
246 247 248	C A C	C A C	C A	C A	C A	C A	G	C/G R	Â	N R	<u> </u>	N N
249 250	C A	c	c	c	c	c	G G	c/G	Ţ	N	Č	N N
251 252	Â	A A T	A A T	A A T	A A T	A A T	G T G	R A/T N	G A G	R A/T N	C A	N A/T N
253 254	G T	Ğ	Ġ T	Ġ	Ġ	Ġ	G T	Ğ	G	Ğ	Č T	G/C Y
255 256	G G	G G	G G	G G	G	Ġ G	Ċ	G/C G	Ċ	G/C G	Ġ	G/C G
257 258	A G	A G	A G	A G	A G	Ā	A C	A G/C	Ā	A G/C	Ā	A G/C
259 260	G T	G T	G T	G T	G T	G T	G T	G T	A C	R Y	C	N N
261 262	A C	A C	A C	A C	A C	A C	G T	R Y	G T	R Y	C A	N N
263 264	Č.	Č.	Č.	C C	C C	t C	T G	T C/G	t C	T C/G	T T	T N
265 266 267	A	A	A	A.	A A	A A	A G	A R	A	A R	A	A R
268 269	A G C	A G C	A G C	A G C	A G C	A G C	G G	R G	G G	R G	G A	R R
270 271	č	Č A	Č A	C A	Č A	C A	A G A	N C/G A	Ğ A	c/G	C G	N C/G
272 273	Ã G	Â	Ã G	Ã G	Ã	Â	Ā	A A G	A A G	A A G	A A	A A R
274 275	Ç T	Č T	č	č	č	Č T	Ğ	c/G T	T T	N T	Î	N T
276 277	G A	G A	G A	G A	G A	G A	G A	G A	G A	Ġ A	Ċ A	G/C A
278 279	A G	A G	A G	A G	A G	A G	G G	R G	C G	N G	A G	N G
280 281	C A	C A	C A	C A	C A	C A	A A	N A	A G	N R	G A	N R
282 283	T G	T G	Ţ G	T G	T G	Ţ Ģ	G G	N G	G T	N N	A A	N N
284 285	T G	T G	T G	T G	Ğ	Ğ.	T C	G/C T	G A	N	A C	N N
286 287 288	GC	G C	G	G C	G C	G C	ç	R C	C A	N N	Č.	N N
289 290	C A G	A G	C A G	C A G	C A	C A G	T G A	Y R R	A	N R	Ğ	N R
291 292	č	c	č	c	G C	c	Ĉ	C A	C G A	N C/G	A G G	N C/G R
293 294							Â	Â	Ť	A A/T C/G	A T	A/T N
295 296	G A	G A	G A	G A	G A	G A	Ğ	Ğ A	Ā	R A/T	Ť C	N N
297 298	C G	C G	C G	C G	C	C G	C G	C G	G A	C/G R	G	C/G R
299 300	A	A	A	A A	A A	A A	A A	A	A G	A R	A G	A R
301 302	G A	G A	G A	G A	G A	G A	G A	G A	A	R A	A	R A
303 304 305	G G	A G	G G	A G	G G	R G	A A	R R	A G	R R	G G	R R
306 307	T G G	T G G	T G G	T G G	ţ G G	T G G	C T G	Y N G	G G	N N	T C	Y N
308 309	A C	A C	A C	A C	A C	A C	A G	A C/G	A G A	R R N	G A T	R R N
310 311	Ā	Ä	Ā	Ä	Ä	Ā	Ä	A A	Ã	A R	Ġ A	R R
312 313	G A	G A	G A	G A	G A	G A	A	R A	A T	R A/T	C	N A/T
314 315	T C	T C	T C	T C	T C	T C	T C	T C	T G	T C/G	T G	T C/G
316 317	G T	G T	G T	G T	G T	G T	A T	R T	C C	N Y	G T	N Y
318 319 320	G C	G C	G C	G C	G	G C	C A	G/C N	G A	e/c	G G	N N
321 322	A G A	Ğ A	A G	A G	A G	A G	A T	Å	G A	R N	c c	N
323 324	A G T	Ä G T	A G T	A G T	A G T	Ä	A A G T G C G C C G T	A A G T	A G T	A R	Ä	A R
325 326	T G	Ť G	T G	Ť	Ť	Ť	ī G	T G	G	N N G/C	Ť	N N G/C
327 328	G	C G	C G	C G	C G	C G	Ċ	С	A C	N	Ť G	N G/C
329 330	T G	Ť G	T G	T G	T G	T G	C	G Y G/C	G G	G/C N G/C	c	N G/C
331 332	G T	G T	G T	G T	G T	G T	G T	G/C G T	Ť G	N N	G T	N N
333 334	C A	C A	C A	C A	C A	C A	C A	C A	A C	N N	C A	N N
335 336 337	Ĝ	Ğ	Ğ	Ĝ	Ĝ	A G	C A G A G	A G	Ğ	N G	A G	N G
337 338 339	G	G C G T G G T C A A G G G C C A C C C C C C C C C C C	G	G C G T G G T C A A G A A G G C C C A C C C A G A G G G C	G C G T G G T C A A G A A G G C C A C A C C A G A G G G	A A G T G C G T C A A G G C C C C C C C C C C C C C C C	G	C A A G A R R G	G C A C G G T G A C T G A A C A C A C T C C C G G A A G A T	GIC N N N N N G A R N R N N N N N N N N N N N N N N N N	A A A T UT T U U U U U T U A A U A A U U A U T U A U T U U T U A U U U T U A U U U T U A U U U U	NG GIG NG NN N
340 341	960	G C	G C	G	G	G	A G A T	G N	Ā	8 R 2	G A	N R
342 343	Č A	Č A	Č A	Č A	C A	Č A	Ť	N Y A C	Ā	N N	Ĉ	N N
344 345	C A	C A	C A	C A	C A	C A	A C T	C A/T	Ť	Y N	c T	Y N
346 347 348	C C	C C	C	C C	C	C C	G T	C/G Y	ć	C/G N	C	C/G N
349	A G	A G	Å Ģ	A G	A G	A G	T G	A/T G	G A	N R	T C	N N
350 351	A G	A G G G	A G	A G	A G	A G G	A A G	A/T C/G Y A/T G A R G	A G	C/G N N R A R	A G	A R
352 353	G A	G A	G A	G A	G A	G A	G A	G A	A T	R A/T	C A	N A/T

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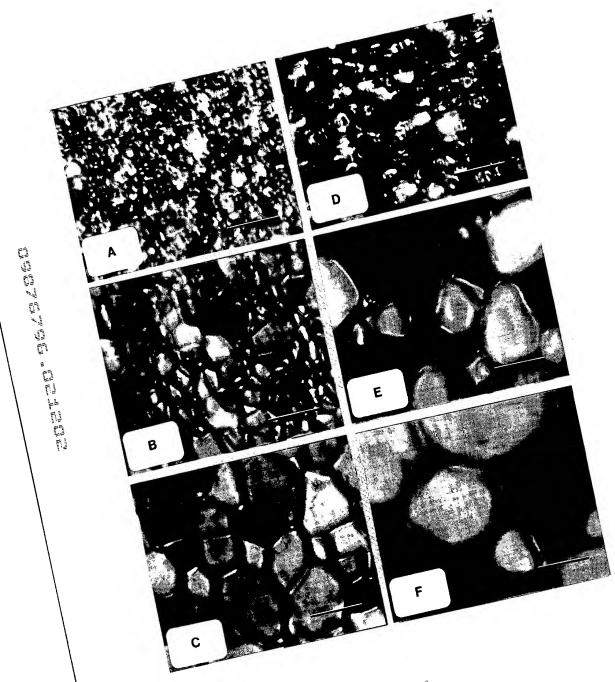


Fig. 8.0



Fig. 8.1a

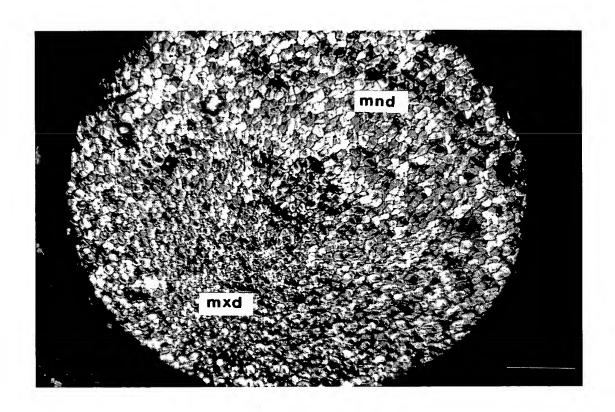
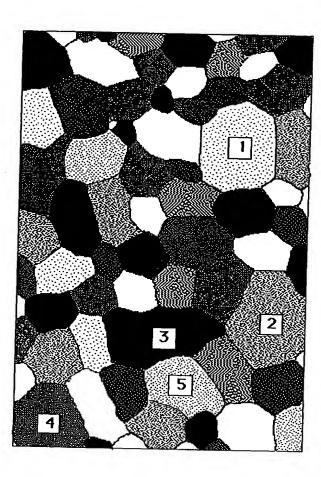
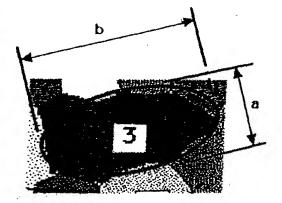
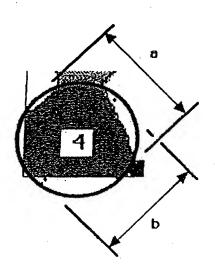


Fig. 8.1b





grain area=0.25mab



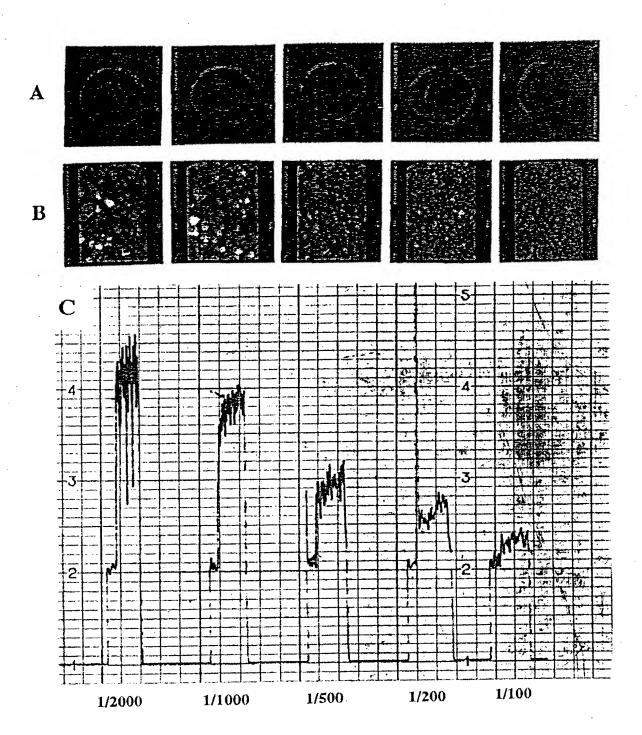


Fig. 8.3

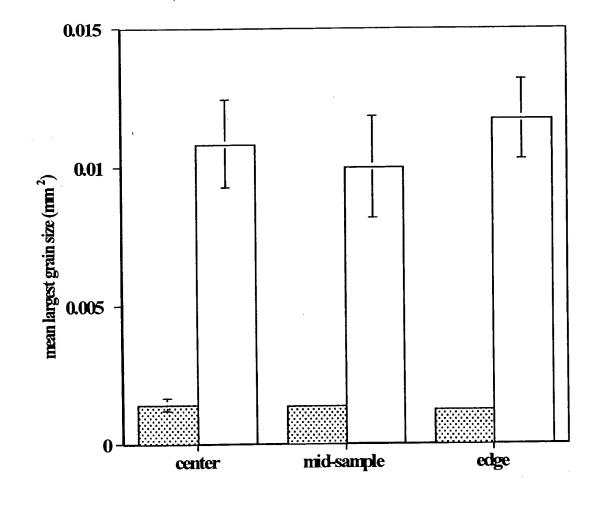


Fig. 8.4a

category

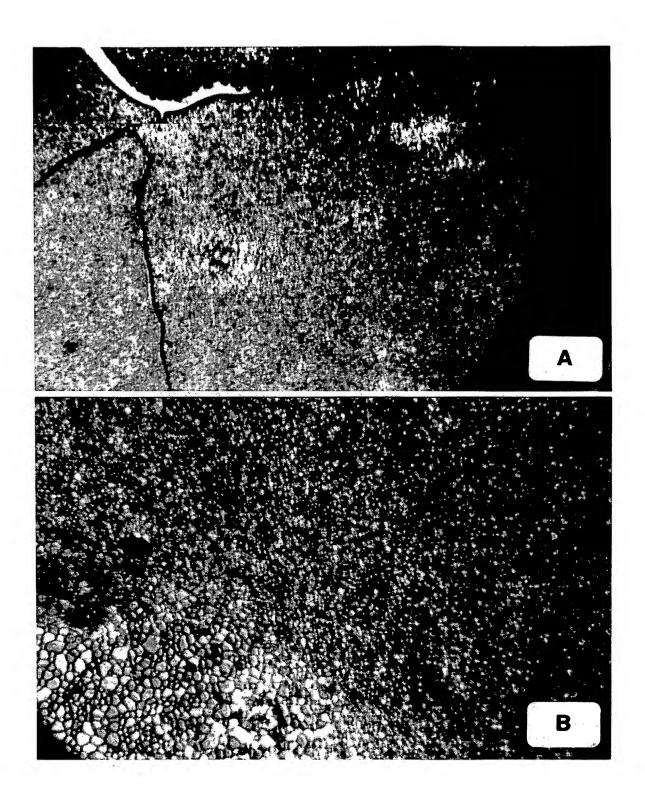


Fig. 8.4b

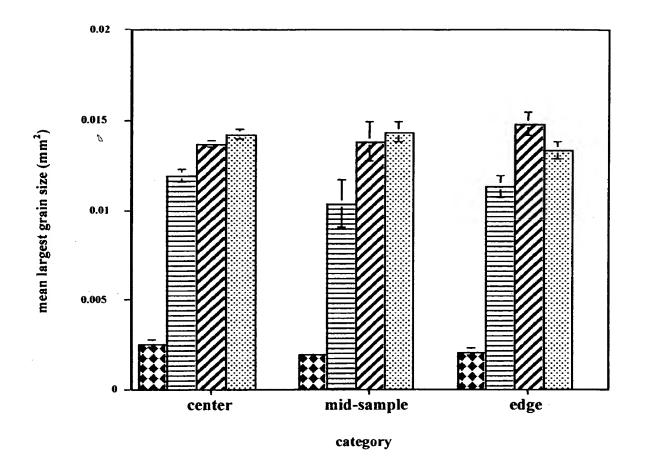


Fig. 8.5a

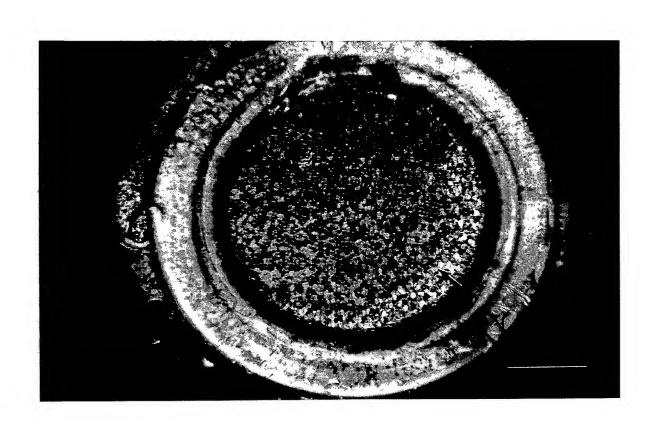


Fig. 8.5b

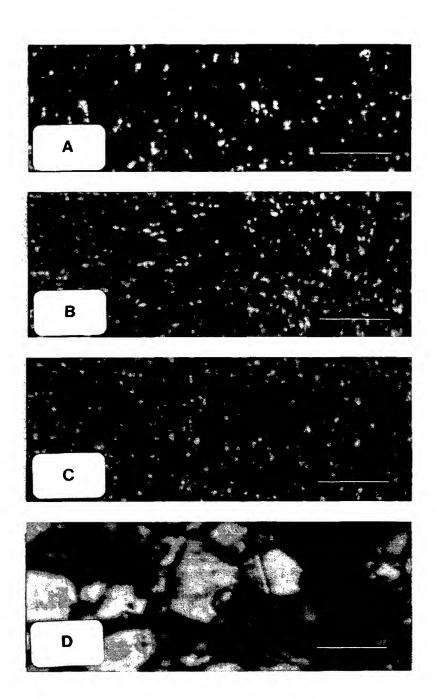


Fig. 8.6

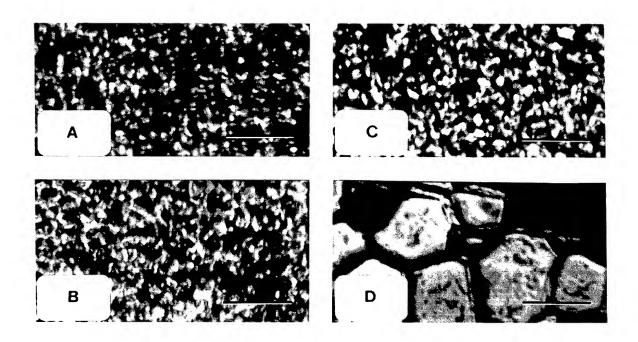


Fig. 8.7

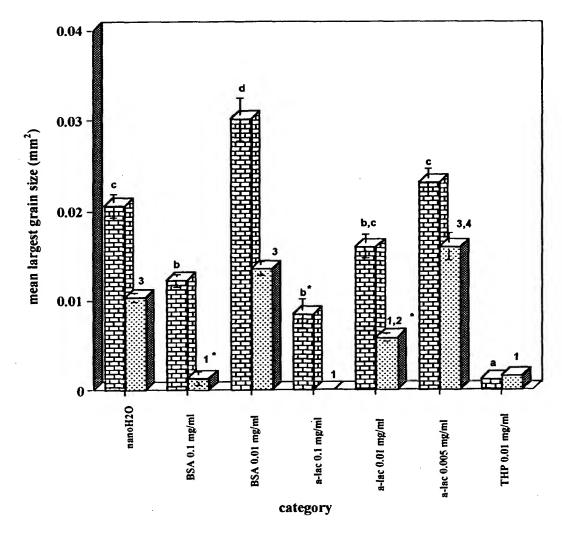


Fig. 8.8

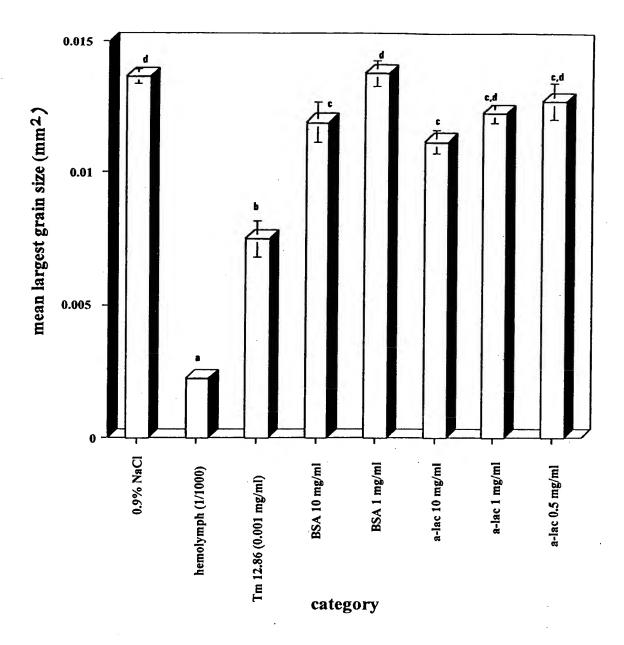
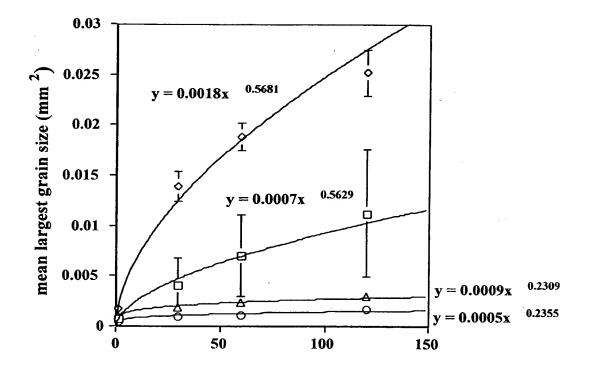


Fig. 8.9



time (minutes)

Fig. 8.10

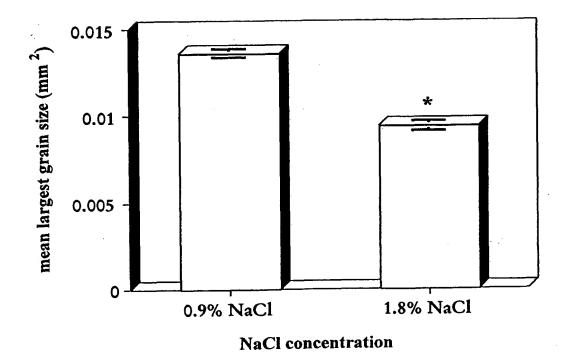


Fig. 8.11

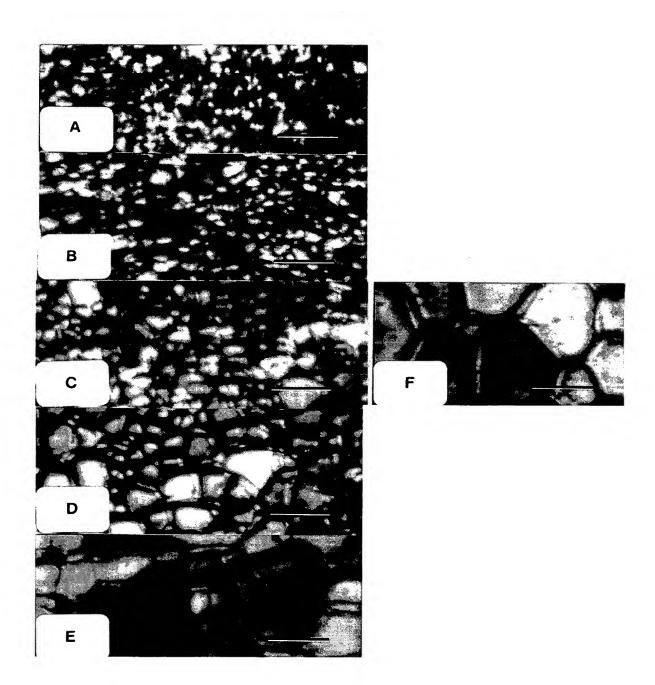


Fig. 8.12

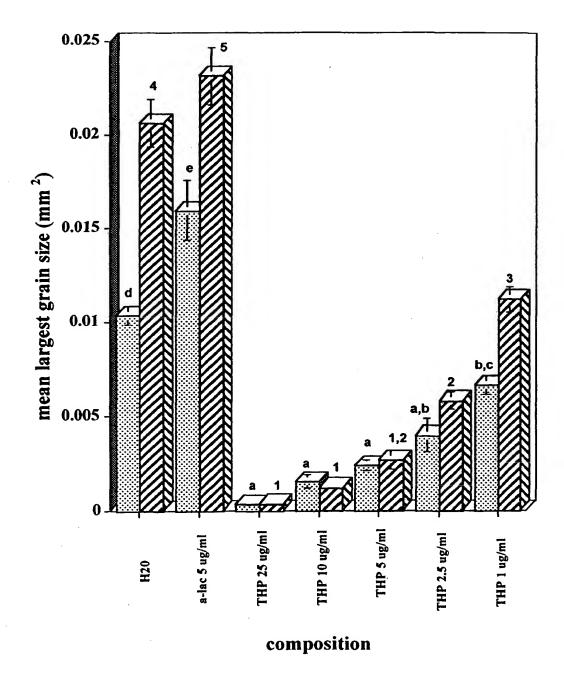


Fig. 8.13

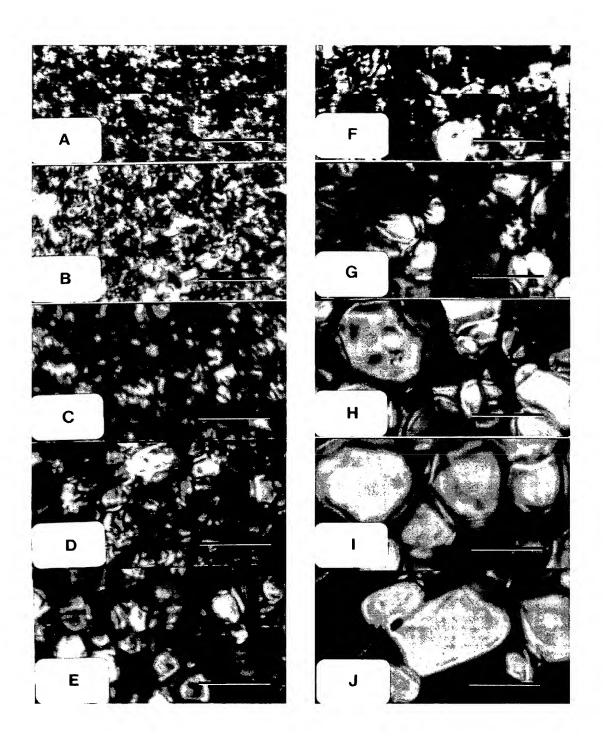


Fig. 8.14

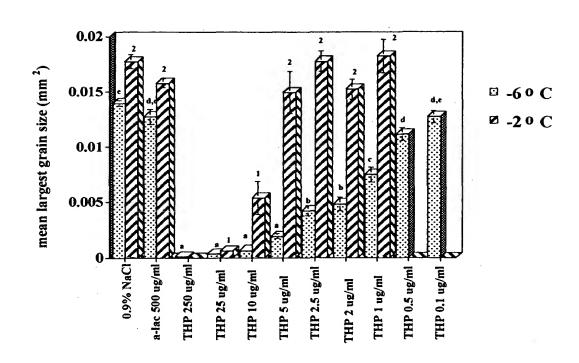
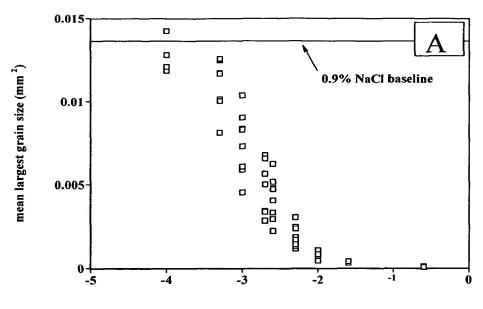


Fig. 8.15



log(Tm 12.86 concentration)

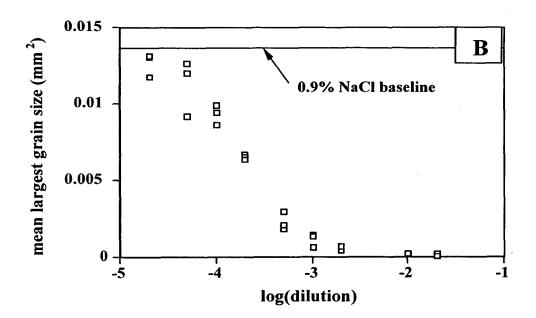
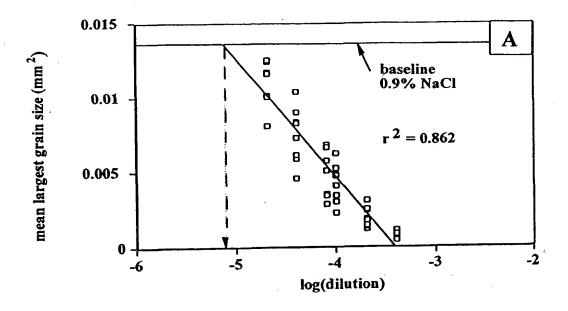


Fig. 8.16



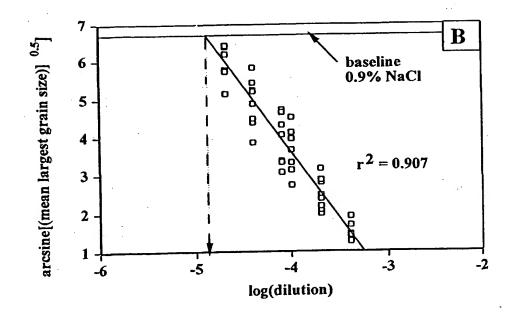
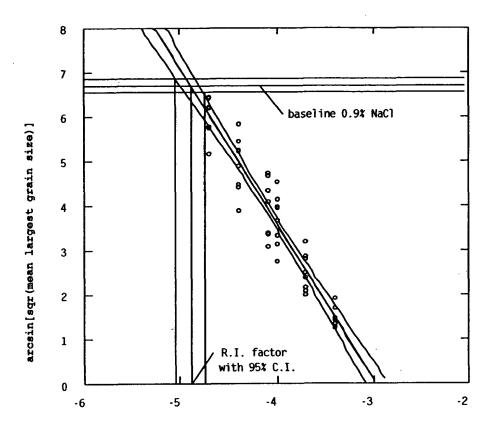
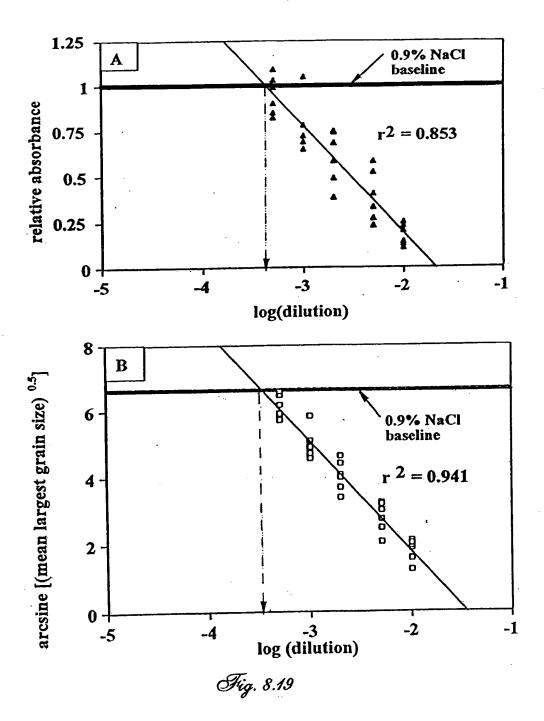


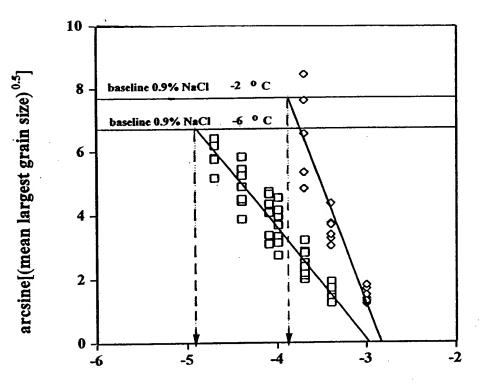
Fig. 8.17



log(dilution)

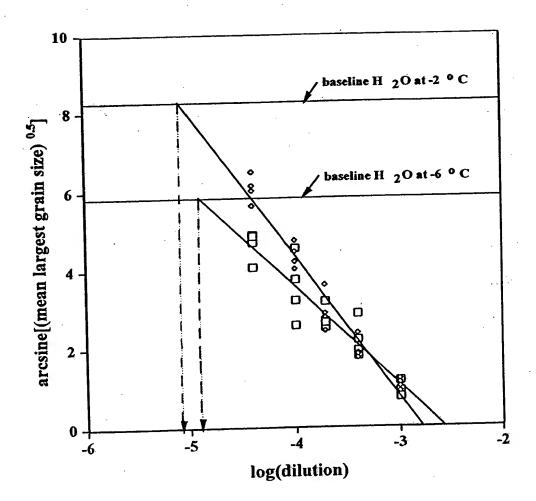
Fig. 8.18





log(dilution)

Fig. 8.20



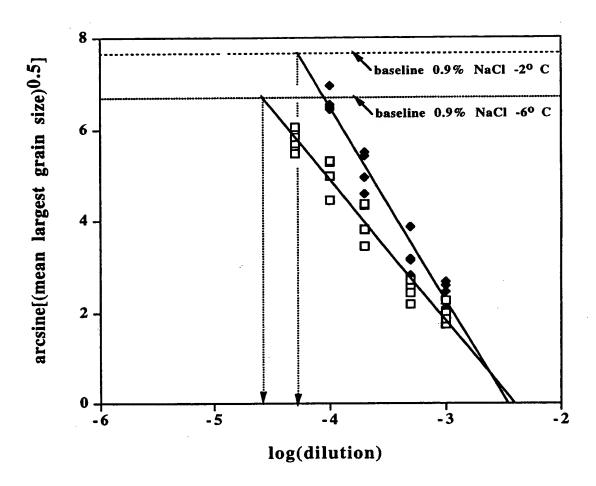


Fig. 8.22

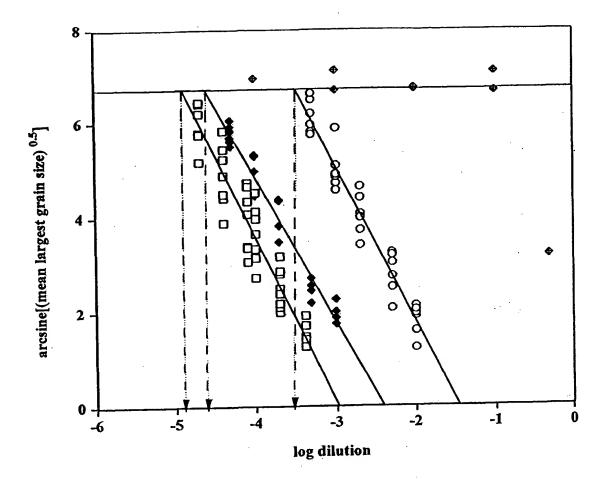
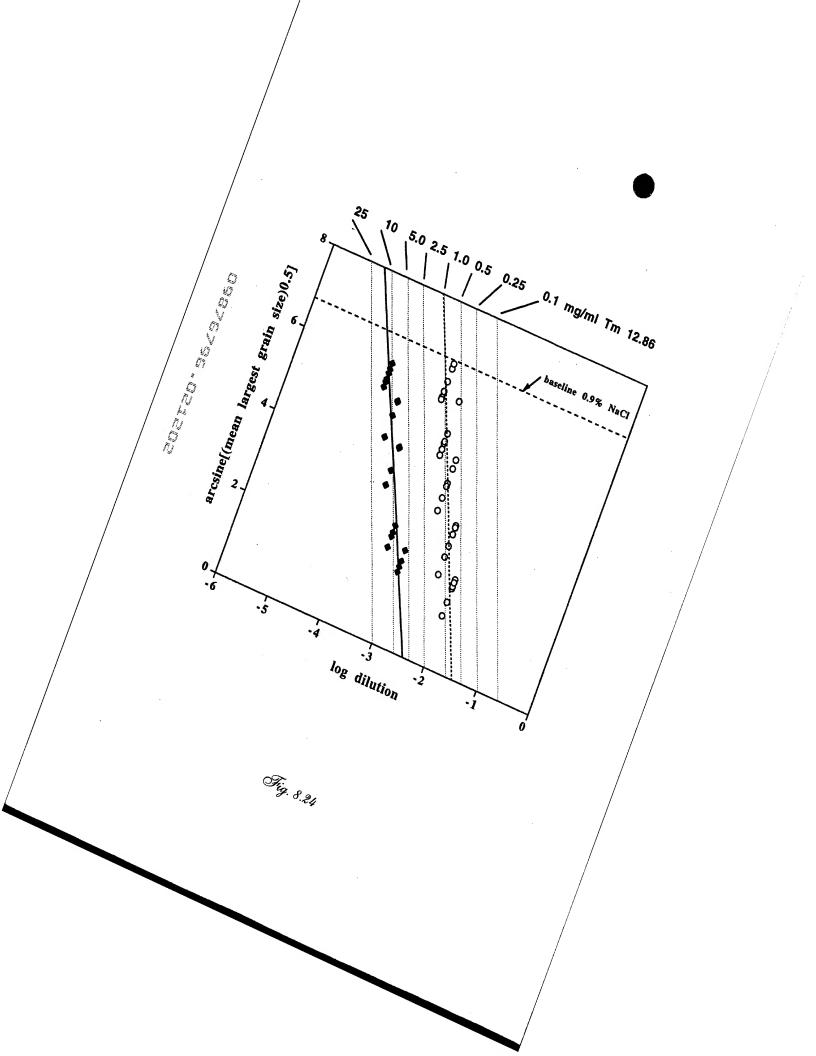
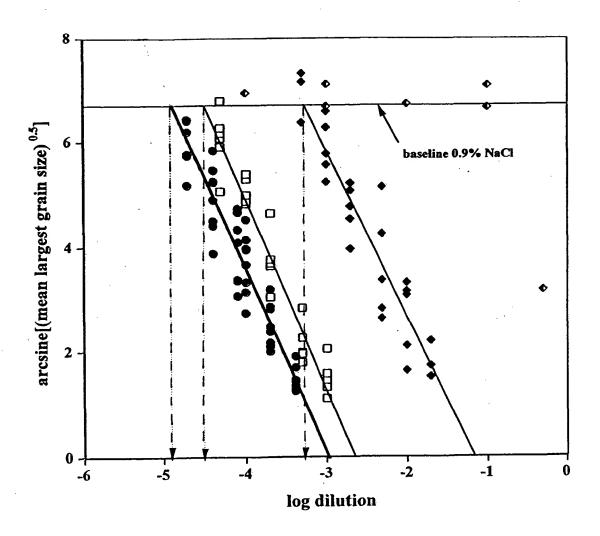
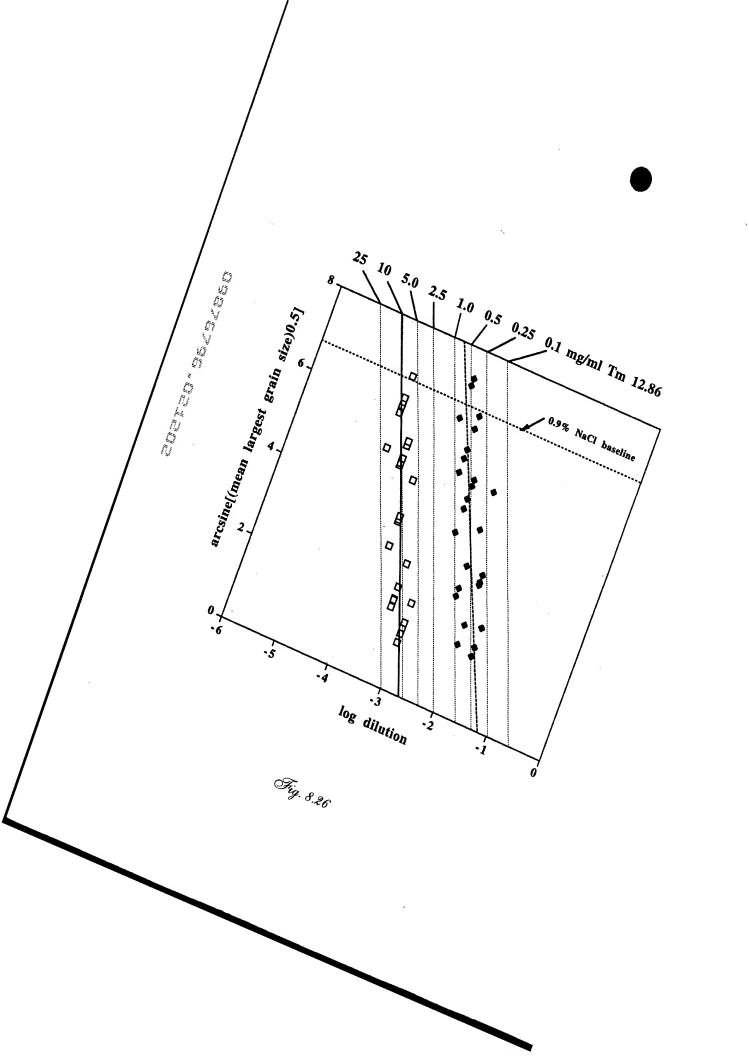


Fig. 8.23







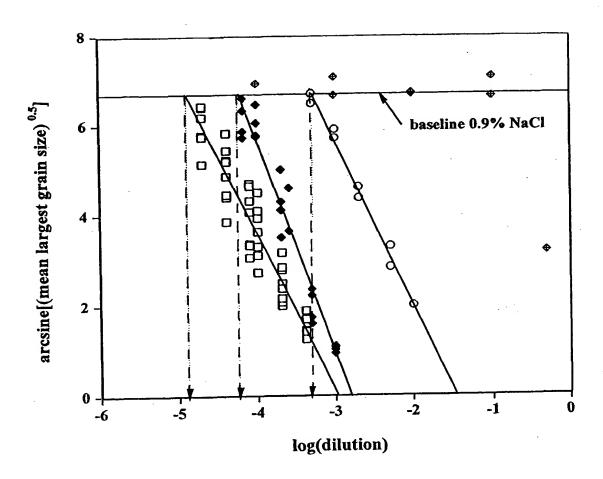


Fig. 8.27

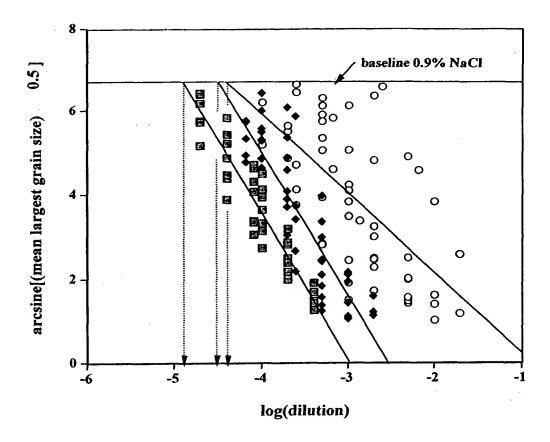


Fig. 8.28

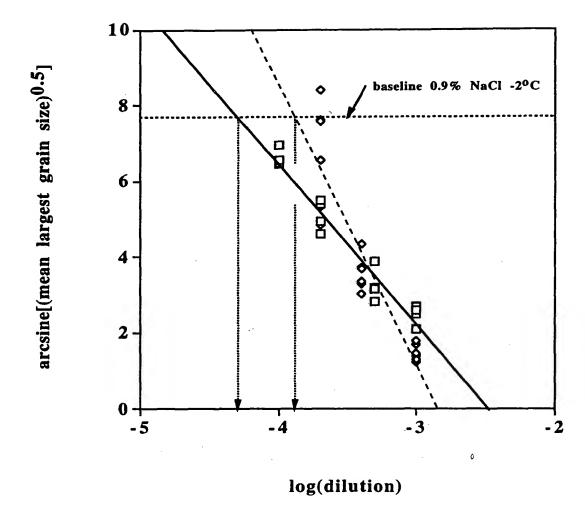
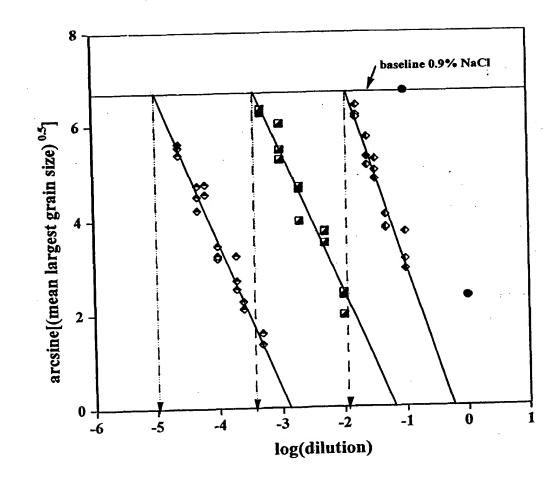
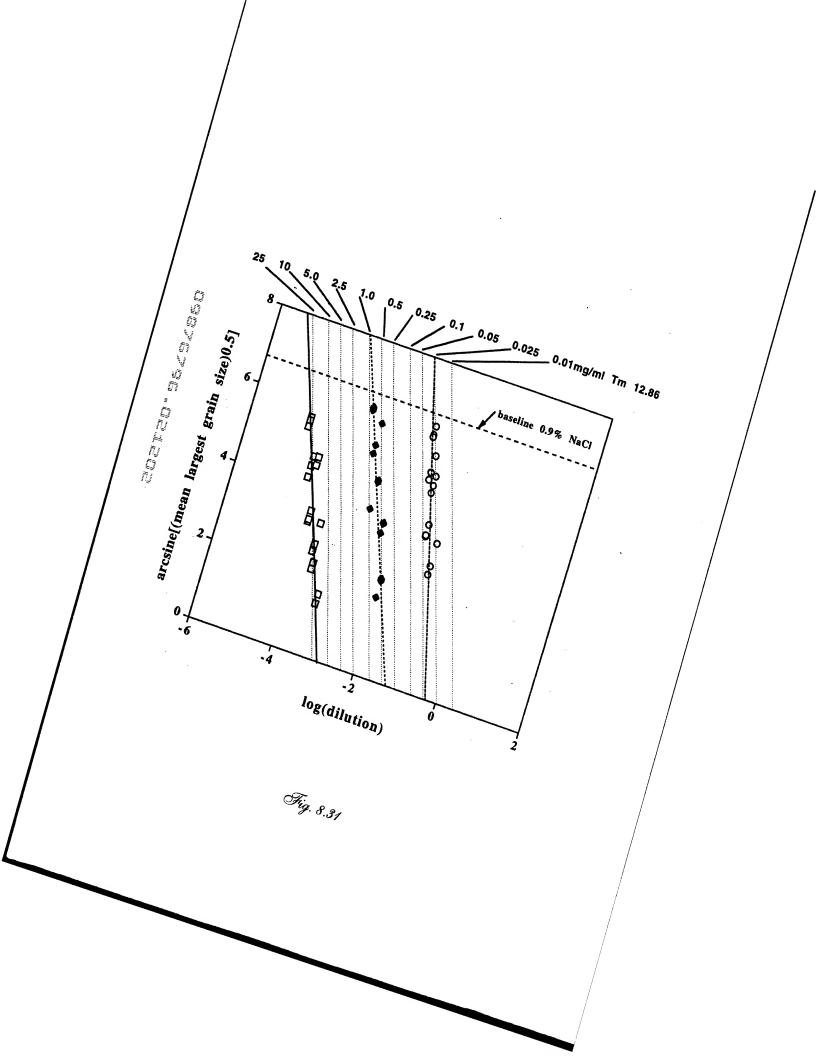
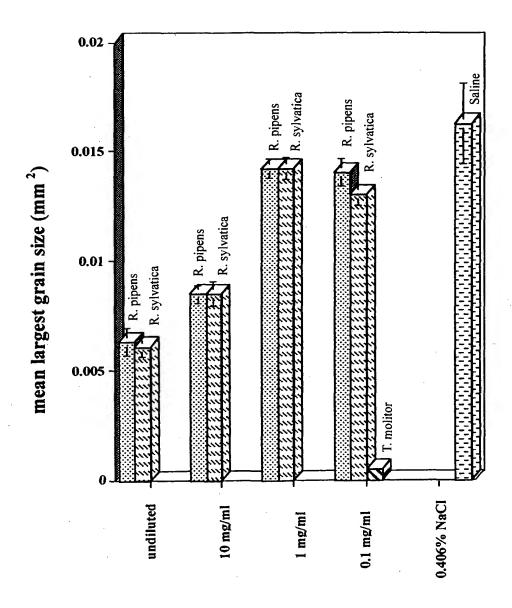


Fig. 8.29







dilution

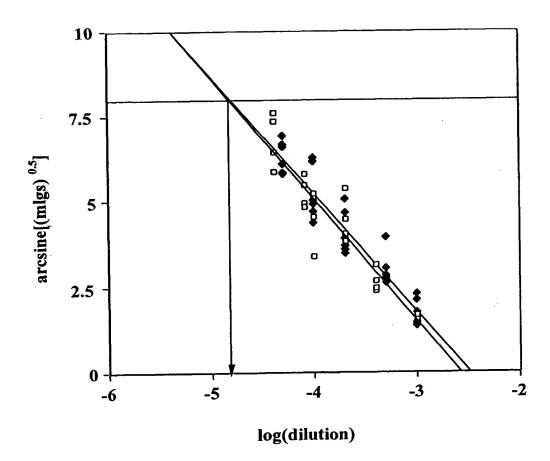
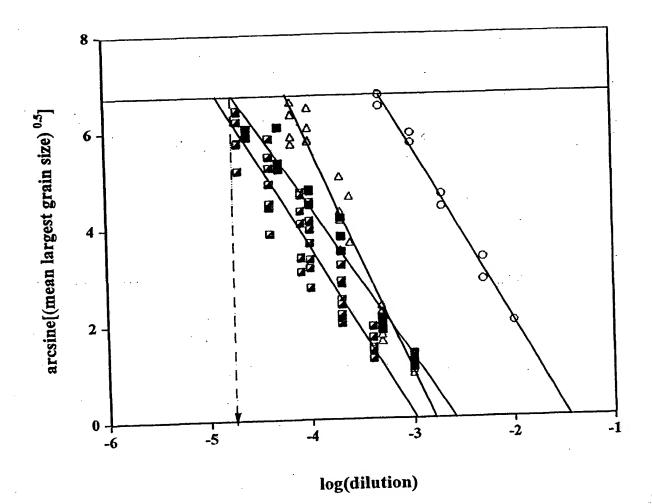


Fig. 8.33



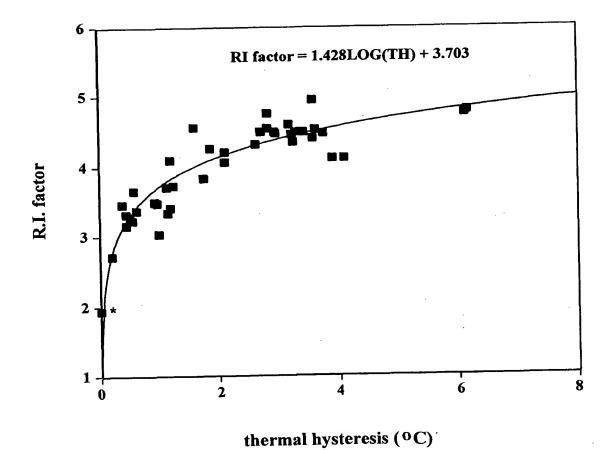
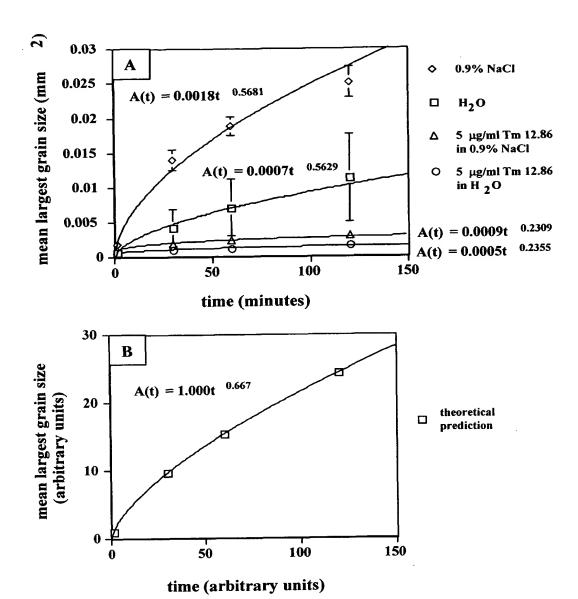
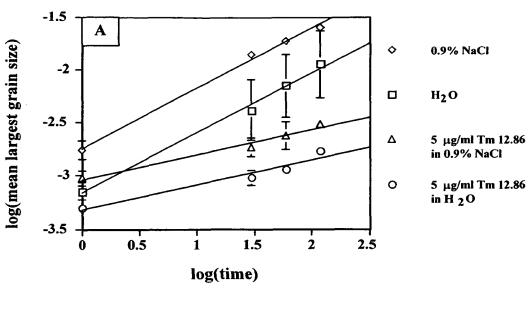
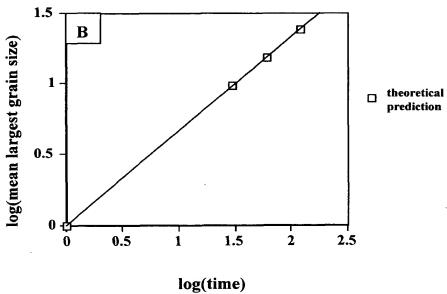


Fig. 8.35







"Sandwich" method of R.I. assessment

1.

10 mm diameter cover glass

liquid samples (~0.1-0.2μ)

3. FREEZE ON ~-80 C ALUMINUM PLATE (~10 MIN.)



4. PLACE ON COLD STAGE, ANNEAL AT -6.C UP TO 12+ HOURS

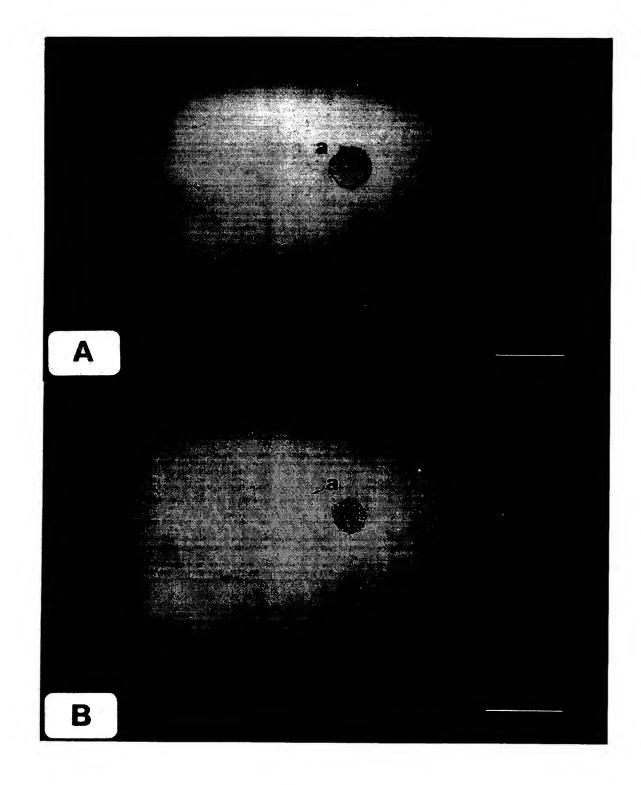


Fig. 8.39

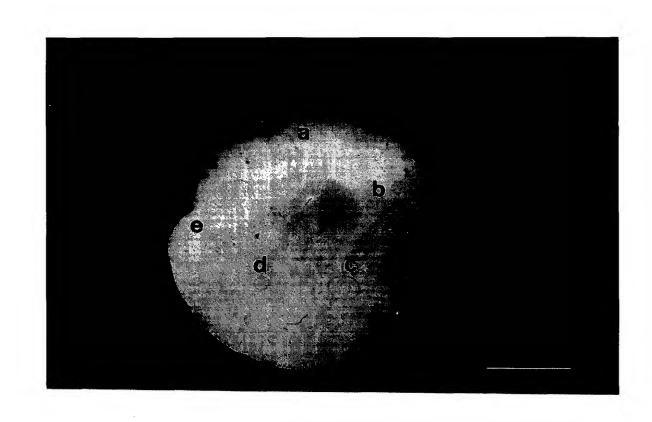


Fig. 8.40

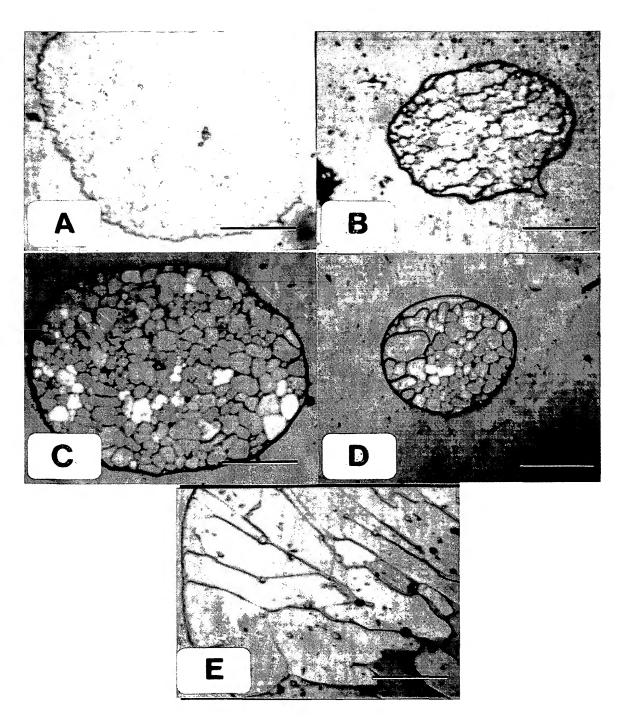
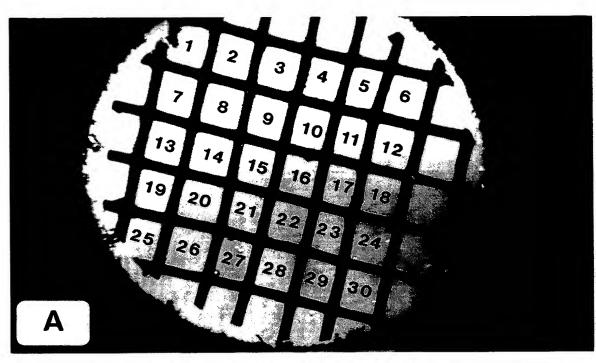


Fig. 8.41



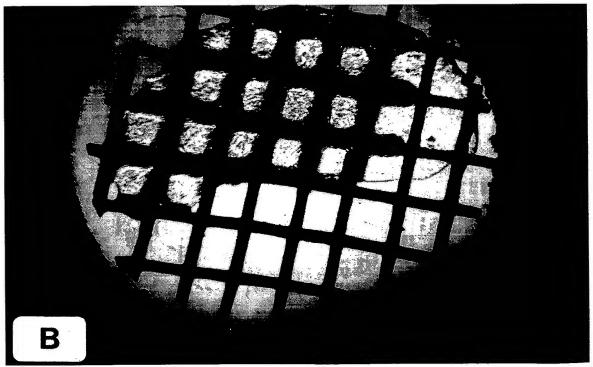


Fig. 8.42

DNA sequence of Tm 13.17 cDNA clone

	В			E																
	а			C																
	m			0		•														
	H			R																
	I			I																
1	AGTG	GAT	CCA	AAG	TAA	TCG	GCA	.CGA	GAC	TAC	TAA	GAT	AA	GTT	GCT	CTG	TTG	TCT.	AAT	CT
												M	K	<u>L</u>	L	С	C	<u>L</u>	I	<u>s</u>
												G				Р				
61	CCCT	CAT	TCT	GTT	GGT	CAC	AGT	TCA	GGC	COI	GAC	CGA	GGC	ACA	AAT	TGA	GAA	ACT	GAA	CA
									<u>A</u>		T		A	Q	I	E	K	L	N	K
									•	T		Y	-							
121	AGAT	CAG	CAA	AAA	ATG	TCA	AAA	TGA	AAG	TGG	AGT	GTC	GCA	AGA	GAT	CAT	AAC	CAA	AGC	TC
***	I		K		С		N	E	S	G	V	S	Q	E	I	I	T	K	Ą	R
1.81	GCAA	CGG	ТGA	CTG	GGA	GGA	CGA	TCC	'TAA	ACT	'GAA	ACG	CCA	AGT	TTT	TTG	CGT	GGC	CAG	GA
	N		D		E	D	מ	P	K	L		R	Q	V	F	C	V	A	R	N
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241	ACGC	cee	መረግጥ	ccc	CDC	מבוצוי	<u>አ</u> ሞር	'CCC	aga:	GGT	GGT	GGT	CGA	CGT	GTT	GAG	GGA	GAA	GGT	GA
741		_		A	T	E	s	G	E	v	v	v	D	V	L	R	E	K		R
	A	G	נג	A		13	U			•	•	•		-	P					
201	GGAA		~ ·	100 C 70	~~ ~	CCT	CCI	ממו	A B C	מכוחיי	GAD	TAA	CAT	CAA		GTG	CGC	CGT	CAA	GA
301					N	D	E	E.	TACE C	E	K	T	T	N	ĸ	С	A	v	ĸ	R
	K	V	T	D	N	ט	E	Ľ		13	11		-	**	~~	_		·		
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361	GAGA												C	A.	M	K	N	K	P	K
	D	T	V	E	E	T	V	F	N	T	F	K	C	¥	M	I.	14		Z,	20
				G													-cmc	mee	en ma	13 C
				12 MM	ነጥርኒክ	ኒጥጥር	SAAC	CAC	CAC	GAC	TAC	FTAG	ATC	1.1.5	CAB	ATG	GIG	TGC	.1.1.1	AC
421	AGTT	CTC	ACC	WGI							•									
421	AGTT F	CTC S	ACC P	V	D	*					,									
421						*					•								×	•
421						*					•								h	ì
421						*													h	i >
		S	P	V	D	*													h	,

polyadenylation signal poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC